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2878

Observations on effects of iodine administration in dogs following hemithyroidectomy and unipolar ligation.

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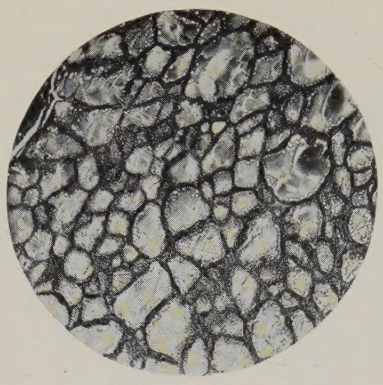
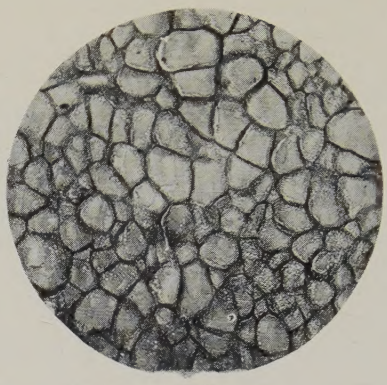
In a previous communication before this society, it was reported that hyperplasia of the acinar cells was frequently observed in the dog following the removal of one lobe and the ligation of the superior pole of the remaining lobe. During the past year these experiments have been continued under similar experimental conditions, but, in addition to hemisection and ligation, iodine was administered either in the food or by subcutaneous injection as sodium iodide in 10 per cent solutions. Excepting in the first three animals, 10 cc. of the solution was given by subcutaneous injection daily for the post-operative period of 5 to 38 days. The experimental animals were mongrels of medium size, of probable mean weight of 7 kilograms. The data on the accompanying table is incomplete, but in so far as one can estimate from the available material, hyperplasia did not develop in this series of postoperative animals. Compared with the forego-

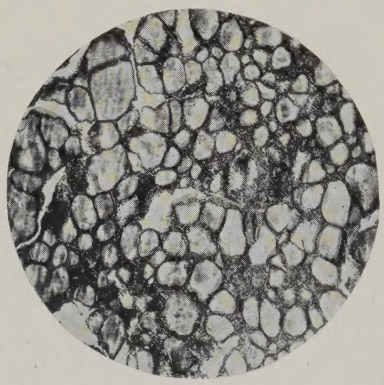


19 PRE-OP. THYROID.

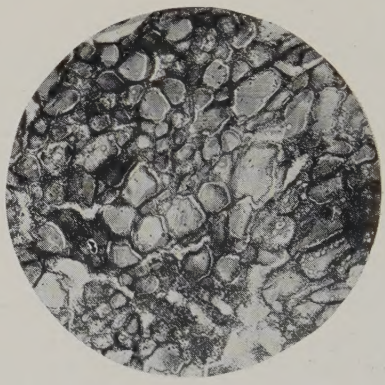


19 POST-OP. THYROID.

No. 19. Thyroid before and after lobectomy and iodine administration showing colloid-filled acini without hyperplasia 23 days after operation. Superior polar vessels were ligated at time of thyroidectomy.



60 PRE-OP. THYROID.



60 POST-OP. THYROID.

No. 60. Thyroid before and after lobectomy and iodine administration, showing colloid accumulation and similar sections 31 days after operation. Superior polar vessels were ligated at time of thyroidectomy.

ing series in which iodine had not been given, the hyperplasia is much less evident; in a few the activity of the acinar cells is less marked than before lobectomy; and in nearly all, the acini appear well filled with colloid. These observations appear to corroborate those reported by Marine (Harvey Lectures, 1924-25) and to corroborate the contention that iodine administration not only causes a diminution of hyperplasia in simple goitre, but, when given in proper dosage, prevents hyperplasia.

Thyroid from normal dog		Thyroid after hemithyroidectomy, unipolar ligation and iodine administration
Exp No.		
19 Normal or slightly hyperplastic	after 23 days	Normal
20 Slightly hyperplastic	7 "	Normal or slightly hyperplastic
22 No specimen	?	Normal
25 Normal or slightly hyperplastic	?	No specimen
32 No specimen	?	Normal
33 Slight hyperplasia	?	Normal
35 Slight hyperplasia	7 "	Normal
37 No specimen	10 "	Normal
38 Hyperplasia	?	Hyperplasia (less)
40 Normal	?	Normal
46 Hyperplasia	5 "	Hyperplasia
51 No specimen	5 "	Normal or slightly hyperplastic
52 Normal or slightly hyperplastic	38 "	Normal or slightly hyperplastic
60 Slightly hyperplastic	31 "	Normal or slightly hyperplastic

2879

Comparison of non-irradiated and irradiated cholesterol to inhibit the hemolytic action of digitonin.

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As is well known, digitonin exerts a hemolytic action on red blood corpuscles, and cholesterol has the power to inhibit this activity. In view of the fact that our experiments have shown that the chemical properties of cholesterol are altered in various ways as the result of irradiation with ultra-violet light, it seemed worth investigating whether its property of inhibiting the action of digitonin remained unchanged after irradiation.

A comparison was made of the effect, in relation to hemolysis, of ordinary cholesterol with that of cholesterol which had been irradiated for periods of ½, 2 and 10 hours with the radiation of a mercury vapor lamp, set at a distance of 1 foot. For this purpose a suspension of 1 per cent ethereal solution of cholesterol and 0.1 per cent digitonin was tested on the red cells of the dog and of the sheep. Without going into detail at this time, it may

be stated that it was found that the rate of speed with which the cholesterol bound the digitonin had been altered as the result of irradiation—that the irradiated cholesterol bound digitonin more readily than ordinary cholesterol, and brought about a comparative delay in its hemolytic action. For example, whereas under controlled and constant conditions ordinary cholesterol allowed complete hemolysis to take place immediately, when cholesterol was used which had been irradiated for 2 hours, complete hemolysis did not occur until after an interval of 1½ to 3 minutes. The sterol-digitonin was incubated for 3 hours before it was added to the red cells. When, however, the cholesterol was irradiated for a prolonged period, for 10 hours, this delay did not take place and complete hemolysis occurred immediately. In this connection it is of interest to note that the various other characteristics acquired through irradiation, for example its antirachitic potency, are lost when the raying is continued for 10 hours, or even a shorter period.

In these experiments the sterol-digitonin mixtures were incubated for 3 to 9 hours. When the incubation was carried out for 9 hours, it was found that the distinction between ordinary and irradiated cholesterol was lost, except in regard to that which had been rayed for 10 hours, which as a rule continued to allow complete hemolysis to take place immediately.

These experiments once more indicate that cholesterol is altered in its chemical constitution as the result of irradiation with ultraviolet rays, and confirm previous experiments in which this phenomenon has been demonstrated by other methods.[1]

[1] Hess, A. F., et al., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 227; PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 319; *J. Biol. Chem.*, 1925, lxiii, 305; *J. Biol. Chem.*, 1925, lxiv, 181; *J. Biol. Chem.*, 1925, lxiv, 193.

2880

Bacillus welchii as an agent in experimental anemia.

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By one or two intramuscular injections of rabbits with 0.5 to 1.0 cc. of whole culture of *B. welchii* it has been possible to produce a severe toxic anemia within five days. The blood picture has been that of a secondary anemia, in which the hemoglobin is reduced to 50 or even to 25 per cent of the normal, and the red blood corpuscles are reduced to 2,380,000 or even 840,000 per cu. mm., accompanied by a leucocytosis of 20,000 to 43,000 per cu. mm. Marked anisocytosis and poikilocytosis were observed at the height of the disturbance. Erythroblasts were observed, from 642 to 4,352 per cu. mm.; among them normoblasts, microblasts and macroblasts. Monocytes or clasmatocytes in the peripheral blood containing ingested red blood cells were present, numbering from 186 to 868 per cu. mm. The maximum loss of weight varied from 9 to 23.9 per cent. Histologic examination of the spleen of these rabbits showed a marked increase of erythrophagocytosis.

Repeated intramuscular injections of 0.1 to 1.0 cc. of whole culture or of toxic filtrates of *B. welchii* into rabbits produced chronic intoxication with accompanying secondary anemia. Those animals treated with whole culture showed decrease in hemoglobin from 56 to 53 per cent of the normal in periods varying from 5 to 19 days. A leucocytosis of 13,000 to 29,400 was present. Anisocytosis, poikilocytosis and polychromatophilia were marked from the third to the fifteenth day of treatment and remained to a slight degree as long as the injections were continued, covering in most instances, a period of 25 to 40 days. Nucleated red cells were present in numbers varying from 148 to 270 per cu. mm. Erythrophagocytosis in the blood stream was not observed. The final loss of weight varied between 8.3 and 8.5 per cent.

Animals treated with toxic filtrates of *B. welchii* revealed a blood picture somewhat less abnormal. The hemoglobin dropped

only to from 82 to 77 per cent of normal and the red blood corpuscles to 3,220,000 to 3,800,000 per cu. mm. Leucocytosis was mild, varying from 10,000 to 21,400. Anisocytosis and polychromatophilia were most marked from the fourth to the tenth day of treatment, diminishing as the injections were continued over long periods. Little or no poikilocytosis appeared except with the use of toxin from one strain of *B. welchii,* in which case this change was marked from the 4th to the 11th day. Erythroblasts and erythrophages were not observed.

Bacteria of two other species common in chronic intestinal disease, namely, *B. bifermentans* and *B. sporogenes,* have been used by us in experimental infection by the same procedure. A mild anemia was produced with *B. bifermentans,* in which the hemoglobin was reduced to from 76 to 73 per cent, the red blood cells to 3,360,000 to 5,880,000 together with a leucocytosis of 17,600 to 19,000. Moderate anisocytosis and slight poikilocytosis appeared. Erythroblasts were seen in the blood of some of these animals, reaching 352 per cu. mm. at one time. No erythrophages could be demonstrated in preparations of peripheral blood. The loss of weight at the height of the infection varied from 17.9 to 44 per cent.

When infection was produced with *B. sporogenes* the hemoglobin showed a slight rise of 3 per cent and the red corpuscles, after an initial loss of 500,000 per cu. mm., rose again to 6,500,000. The leucocyte count reached 15,200. A moderate anisocytosis and polychromatophilia were observed but no erythroblasts nor erythrophages could be demonstrated by the usual technic. There was no appreciable change in weight.

Since butyric acid is a product of *B. welchii,* a one per cent solution of it has been administered by intramuscular injection, seventeen such injections being given over a period of 44 days. Hemoglobin diminished to 56 per cent of the normal and the red cells decreased to 2,760,000. Marked anisocytosis and poikilocytosis were present over half the period covered by the treatment. Nucleated red blood cells appeared on the thirtieth day, numbering 64 per cu. mm. The loss of weight was 18 per cent.

Sterile distilled water was also used as a hemolytic agent, injections being given intravenously for 19 days. The red cell counts, made approximately 30 minutes after each injection,

showed a minimum count of 2,644,000. This destruction was compensated, however, in a considerable measure before the next injection, the lowest count observed immediately before injection being 3,220,000. Anisocytosis and polychromatophilia became marked on the 17th day. Erythroblasts appeared on the 12th day, numbering 124 per cu. mm. The loss of weight was 36 per cent.

The production of more or less severe experimental anemia in animals by injections of bacterial toxin only verifies generally accepted clinical experience on human beings that such poisons play a very important part in the more chronic types of anemia of man, but it is evident that further observations will be required before one can justly designate such experimental anemias, even when they are very severe or fatal, as presenting the counterpart of so called pernicious anemia in man.[1, 2]

2881

Lactic acid and inorganic phosphorus of normals and diabetics after glucose, with and without insulin.

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Briggs, Koechig, Doisy and Weber[1] have observed a decrease in sugar, inorganic phosphorus, and potassium of the blood of normal dogs after insulin. There was a parallel increase in the lactic acid apparently formed from the glucose under the influence of insulin. The animals were not anesthetized, but the authors believe that the increased muscular activity played no part in the observed production of lactic acid. Best and Ridout[2] state that the blood lactic acid of dogs does not significantly increase during

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1 Cornell, Beaumont S., *J. Infect. Dis.*, 1925, vi, 508.

2 Kahn and Torrey, PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 8-13.

1 Briggs, A. P., Koechig, I., Doisy, C. A., and Weber, C. J., *J. Biol. Chem.*, 1924, lviii, 721.

2 Best, C. H., and Ridout, J. H., *J. Biol. Chem.*, 1925, lxiii, 197.

insulin hypoglycemia, when this condition is uncomplicated by extreme asthenia or by marked hyperirritability. Moreover Cori[3] has found that insulin hypoglycemia produced no definite change in the lactic acid content of the blood of either fasting rabbits or cats. Nor did the insulin have any effect on the blood lactic acid of phlorizinized rabbits or depancreatized cats, but the insulin convulsions lead to a strong increase in the lactic acid concentration of the blood. Blatherwick, Bell and Hill[4] observed in normal individuals a marked decrease of the inorganic phosphorus of blood plasma accompanied by a lessened excretion of phosphorus in the urine after the administration of insulin to normal individuals. These changes occur during the period of hypoglycemia. A comparison of *in vitro*-glycolysis with the hypoglycemia after insulin has been made by Morgulis and Barkus.[5] These authors state that *in vitro*-glycolysis is different from the hypoglycemia caused by insulin in that the disappearance of the glucose in the former is parallel with the formation of lactic acid, but the insulin hypoglycemia is not necessarily associated with a production of lactic acid.

This brief survey of the literature reveals an apparent contradiction in reported data on changes in the blood lactic acid associated with insulin hypoglycemia. With the object of determining the relation of changes in the lactic acid concentration of the blood to the oxidation or storage of glucose, the sugar and lactic acid of blood of rabbits were studied before and after insulin administration. In these experiments eight rabbits were used (average weight, 2 kilograms). One animal was given 10 units of insulin, and two hours later the blood sugar was reduced to 0.047 per cent, and the lactic acid rose to 300 per cent of the control concentration. These changes accompanied convulsions, during which the animal died. The administration of strychnine sulphate produced convulsions in a second animal resulting in death. In this instance the blood lactic acid was increased at the time of the convulsions to 200 per cent of the control figure. Here, however, the hyperglycemia was observed at the time of convulsions. The six remaining rabbits received sufficient of a 50 per cent urethane solution to produce complete muscular relaxation

[3] Cori, C. F., *J. Biol. Chem.*, 1925, lxiii, 253.

[4] Blatherwick, N. R., Bell, M., and Hill, E., *J. Biol. Chem.*, 1924, lxi, 241.

[5] Morgulis, S., and Barkus, O., *J. Biol. Chem.*, 1925, lxv, 1.

of the extremities. Control specimens of blood were obtained and then insulin administered. The dose of insulin used varied from 2 to 40 units. Hypoglycemia resulted in all cases sufficient to cause death, but no convulsions were observed. The changes in the blood lactic acid were insignificant.

Although Mendel, Engel and Goldscheider[6] report that the usual rise and fall in the blood sugar after the ingestion of 100 grams of glucose occurs independently of any alteration in the blood lactic acid, it has been found that the administration of 1.75 grams of glucose per kilogram of body weight results in an increase in the blood lactic acid in normals, hyperthyroids and diabetics. However, the changes in the inorganic phosphorus, either of the blood or urine were not uniform. Changes in the sugar, lactic acid and inorganic phosphorus of the blood after glucose and insulin have been studied in four normal individuals and six cases of diabetes mellitus. Clausen's method has been utilized for the determination of the lactic acid of the blood. With this method in 78 individuals representing normals and pathological conditions showing no disturbance of carbohydrate metabolism, the maximum concentration of lactic acid was 18 mg. per 100 cc. of blood, the minimum 11 mg., the majority varied from 13 to 15 mg. Venous blood was drawn from the arm without stasis, after the subject lay at rest for one hour in the fasting state. Specimens were analyzed immediately after withdrawal.

The observations were made on the subjects after a night's fast of 12 to 14 hours. For an hour preceding and throughout the entire period of the observations, the subjects were maintained in a state of complete rest in bed. At end of the first hour a control specimen of blood was obtained. Then glucose was given by mouth, 1.75 grams per kilogram of body weight. Specimens of blood were drawn at hourly intervals for 2 or 3 hours, then insulin was given. In the normal cases 10 units of insulin were given, with about 50 grams of glucose by mouth. After the ingestion of the glucose by the normal individuals, the lactic acid rose to 2 to 3 times the concentration of the control blood. The maximum concentration was observed after the blood sugar had begun its return to normal. However, following the administra-

[6] Mendel, B., Engel, W., and Goldscheider, I., *Klin. Woch.*, 1925, iv, 306 and 542.

tion of insulin the lactic acid of the blood rose to 4 to 10 times the control concentration. The rise in the lactic acid paralleled the drop in sugar.

In the diabetic subjects the ingestion of glucose produced an increase in the blood lactic acid of from 50 to 100 per cent of the control concentration. The administration of the insulin resulted in a rise in the lactic acid, paralleling the decrease in the blood sugar. In the cases of diabetes the dosage of insulin was based upon the level of blood sugar. The response of the blood lactic acid to the insulin in diabetes was much less pronounced than in the normal subjects. In both the normals and diabetics, the insulin administration produced a decrease of the inorganic phosphorus of the blood and urine, with a subsequent return to normal. Glycolysis *in vitro* was accompanied by a rise in the blood lactic acid; however this increase is not as great as the rise associated with insulin hypoglycemia. The increase in the lactic acid does not account for the entire loss in blood sugar in either case.

2882

The development of cutaneous hypersensitiveness following the intestinal absorption of antigenic protein.

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It has been shown by Schloss and Anderson,[1] and Anderson and Schloss[2] that marasmic infants frequently absorb antigenic protein from the intestinal tract in amounts sufficient to cause the appearance of specific precipitin in the blood. In many instances also, the blood has the power passively to sensitize guinea pigs to

[1] Schloss, O. M., and Anderson, A. F., Allergy to Cow's Milk in Infants with Severe Malnutrition, PROC. SOC. EXP. BIOL. AND MED., 1922, xx, 5.

[2] Anderson, A. F., and Schloss, O. M., Allergy to Cow's Milk in Infants with Nutritional Disorders, *Am. J. Dis. Child.*, 1923, xxvi, 341.

the specific protein. It seemed of interest therefore to determine whether the enteral absorption of antigenic protein leads also to cutaneous hypersensitiveness.

Tests were made using both the cutaneous and intracutaneous methods. In no case was the reaction by the cutaneous method positive. Accordingly, the data presented in this paper is based solely on the results of the intracutaneous tests.

Solutions of the proteins in physiological saline were made up at frequent intervals. Dilutions of 1:100 to 1:1000 were employed, the strength varying with the different proteins used. Chinosol was added as a preservative. One twentieth of a cubic centimeter of the test solution was injected intradermally over the back, chest, abdomen or forearms. Readings were made at frequent intervals for one hour. Control tests were done with the solvent alone.

A reaction was considered positive only if there was a very marked urticaria-like wheal with definite irregularity of the edges—the so-called pseudopodia. In many cases there was a marked erythema or a larger wheal than shown by the control, but it was deemed wiser to disregard such reactions, and to consider positive only unquestionable reactions.

All of the intracutaneous tests described in this paper were done upon the infants who were tested by Anderson, Schloss and Myers for the presence of precipitin in the blood. As far as possible, both tests were done on the same day so that a comparison of results could be made.

It was deemed of great importance to rule out the possibility of a reaction being due to inherited hypersensitiveness. This was done in two ways. In nearly all of the cases there were repeated negative tests prior to the appearance of the positive reaction. In a few cases where the first tests were positive, subsequent negative ones were always obtained. Babies with eczema were not included.

Twenty-three marasmic infants were tested for cutaneous hypersensitiveness to cow's milk protein. Twelve showed positive reactions. There was a marked variation in the duration of cutaneous hypersensitiveness. It ranged from four days to two months or more. Five cases still showed a positive reaction, when last tested, after having been followed for periods between 2 weeks and 48 days.

In relation to the appearance of precipitins in the blood, it was found that in only one case was the precipitin test negative and the intracutaneous test positive. In eleven cases precipitin was present and the intracutaneous tests were negative. In eleven cases both tests were positive. In these eleven cases, precipitin appeared prior to the skin reactions in seven, subsequent to them in two, and at the same time in two.

These results show that a considerable number of marasmic infants develop cutaneous hypersensitiveness to cow's milk. The skin hypersensitiveness appears in most instances at about the same time that precipitin is demonstrated in the blood.

Further observations were made to determine whether ingestion of protein foods other than milk would give similar results. The first group consisted of 19 marasmic infants who ingested 6 to 12 grams of egg white a day in addition to their regular diet. On these infants, tests for precipitin were made by Anderson, Schloss and Myers. Of these nineteen marasmic infants, fifteen showed positive intracutaneous reactions to egg. There was a great variation in the duration of skin hypersensitiveness in these cases. It lasted from three days to eight months. Five infants still gave positive reactions when last tested, after having been followed from 2 weeks to 43 days. The interval between the first ingestion of egg and the appearance of positive reactions likewise showed a marked variation. The shortest period was six days and the longest seventy-four days, while the average was thirty-three days.

In relation to the appearance of precipitin, it was found that in one case there were positive skin tests and negative precipitin reactions; in three cases negative skin tests and positive precipitin tests; in one, both tests were negative; and in fourteen, both tests were positive. Of these fourteen, precipitin appeared prior to the skin reactions in eight, after them in one, and at the same time in five.

In a third group of cases sheep serum was added to the diet. The total daily amount was 30 to 60 cc. Six marasmic infants were tested and of this number one showed a positive cutaneous reaction. No conclusion as to the duration of this reaction could be drawn as the test was still positive at the end of one month. The reaction appeared thirteen days after the first feeding of sheep serum. Precipitin appeared in the blood in all of the six

cases. In the one case where both tests were positive, precipitin appeared four days after the cutaneous test became positive.

It is thus seen that, following the ingestion of foreign protein, a great number of marasmic infants develop cutaneous hypersensitiveness to the protein ingested. This skin reaction usually occurs either immediately after, or at the same time as, the appearance of antibody in the blood stream.

Further tests were then done on normal infants whose blood was examined for precipitin to determine if they likewise developed cutaneous hypersensitiveness. The first group comprised breast fed infants who, while under observation, were given complementary or supplementary feedings of cow's milk, and also very young infants who had ingested cow's milk for not more than two weeks prior to admission. Ten infants were included in this group. Of this number six showed positive intracutaneous reactions to cow's milk. Precipitin for cow's milk was present in the blood of all six cases, appearing prior to the skin reaction in one case, at the same time in four cases, and afterwards in one case.

A second group of normal infants who were fed egg white, were tested for cutaneous hypersensitiveness to egg. Eight of the 21 infants in this group gave positive reactions. The interval between the first ingestion of egg and the appearance of a positive skin reaction ranged between ten and twenty-eight days with an average interval of seventeen days. The duration of the skin hypersensitiveness varied between ten days and one month. Precipitin for egg was not demonstrated in the blood of two of the infants who developed a positive cutaneous reaction. In six cases both tests were positive. A positive precipitin reaction was obtained first in two cases, afterwards in two cases, and at the same time in two cases.

A very small group of four normal infants received sheep serum in their food. One infant showed a positive skin reaction which appeared forty days after the first feeding. The reaction was still positive two weeks after its appearance, when the last test was done. It appeared at the same time that precipitin for sheep serum was demonstrated in the blood. The small number of positive reactions which occurred after feeding sheep serum to both marasmic and normal infants might possibly be explained by the fact that the actual amount of protein ingested was much less than in the case of milk and egg.

A fourth group of normal infants was fed almond flour. Fifteen to fifty grams were added to the daily feedings. Fifteen infants were tested and of this number two showed positive skin tests. These appeared seventeen and twenty-three days after the first feeding. In both cases precipitin was present prior to the skin reactions.

These results indicate that the enteral absorption of antigenic protein by normal or marasmic infants leads not only to the appearance of a specific precipitin in the blood, but also in many cases to cutaneous hypersensitiveness. The results also indicate that there is a definite coincidence between the appearance of the skin reaction and the presence of precipitin in the blood.

2883

The intestinal absorption of antigenic protein by normal infants.

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In previous communications,[1] it has been shown that the blood of most marasmic infants contains precipitin for cow's milk at some period of the disease, indicating preceding enteral absorption of antigenic protein from cow's milk.

Supported by a large number of observations, it is generally assumed that in the process of normal digestion no antigenic protein product enters the blood. In a few isolated experiments, which involved feeding large amounts of protein at a single dose, absorption of antigenic protein by normal individuals has been demonstrated by Ascoli, Schloss and Worthen, and others. Most investigations of this type, however, have been negative. In a large number of normal infants, tests for precipitin for cow's milk made by us, have been uniformly negative.

[1] Schloss, O. M., and Anderson, A. F., Allergy to Cow's Milk in Infants with Severe Malnutrition, PROC. SOC. EXP. BIOL. AND MED., 1922, xx, 5; Anderson, A. F., and Schloss, O. M., Allergy to Cow's Milk in Infants with Nutritional Disorders, *Am. J. Dis. Child.*, 1923, xxvi, 451.

The fact that precipitin for cow's milk may be present in the blood of marasmic infants for a time but later disappear, despite the continued ingestion of cow's milk, suggested that perhaps a similar process may occur with normal infants. If this were true, normal infants might absorb antigenic protein in amounts sufficient to provoke the production of precipitin for a short time only, in which event negative precipitin tests would not prove that antigenic protein were not absorbed at some previous time. It therefore seemed desirable to observe normal infants whose food contained proteins which they had not ingested before, and to determine whether sufficient antigenic protein was absorbed from the intestinal tract to cause the appearance of specific precipitin. Pursuant of this, observations were conducted on normal infants who received egg white, sheep serum and almond in their food. We have had opportunity also to observe normal infants who received cow's milk for the first time.

The proteins were fed in the following fashion. Six to 12 grams of powdered egg white, 30 to 60 cc. of sheep serum or 15 to 30 grams of almond meal were added to the day's food. The infants received, therefore, a relatively low concentration of the added protein. Precipitin tests on the blood of each patient were made before the special proteins were added to the food, and afterwards, at approximately 72 hour intervals. The precipitin tests were conducted by adding from .1 to .2 cc. of the patients' serum to dilutions of the protein in concentrations from 1:100 to 1:5,000. The concentrations were varied somewhat with different proteins. The usual controls were carried out routinely.

Thirteen normal infants were fed egg white in the quantities mentioned. All of these infants developed precipitin for egg white. In 10 instances the precipitin appeared 9 to 14 days after the addition of egg to the food. In the other 3 cases, it appeared 28, 30 and 40 days after egg was first ingested.

The results from feeding sheep serum were practically identical. The three infants in this group showed precipitin to sheep serum 8, 13 and 19 days respectively after sheep serum was first ingested.

Twelve infants or young children from 10 months to 3 years of age were fed almond meal. Nine developed precipitin to amandin—the globulin of almond—from 9 to 15 days after the special feeding was begun.

Of special interest is a group of 9 infants who were observed when they began to ingest cow's milk. They had previously received human milk exclusively and were either abruptly weaned or were given one or more feedings of cow's milk in addition to human milk. All of these infants developed precipitin for cow's milk at intervals of 17, 12, 16, 13, 25, 15, 10, 8 and 12 days respectively, after the ingestion of cow's milk was commenced.

It is significant that in most of these observations the precipitin appeared promptly, usually in about two weeks. It was of interest also that the degree of precipitin formation was relatively slight and that the precipitin could be demonstrated in the blood for a comparatively short period only.

The phenomenon of precipitin formation which we have observed in normal infants is strikingly different from that observed in marasmic infants. In the latter, the continued ingestion of cow's milk may fail to provoke the production of precipitin until the lapse of many weeks; while in normal infants precipitin appears very promptly after the first ingestion of cow's milk. Furthermore, the degree of precipitin formation in marasmic infants is much more marked than in normal infants, indicating an absorption of relatively larger amounts of antigenic protein. Finally, judging by the duration of precipitin in the blood, marasmic infants absorb antigenic protein from the intestinal tract over a much longer period of time than do normal infants.

These observations demonstrate that when normal infants ingest cow's milk, egg albumin, sheep serum or almond for the first time, sufficient protein is absorbed in antigenic form to provoke the production of specific precipitin in the blood. The early disappearance of the precipitin would seem to indicate that such absorption is of relatively short duration. A consideration of the exact mechanism involved in the absorption of antigenic protein and the cessation of such absorption, would be largely speculative and therefore out of place at this time.

2884

Immunity against pneumococcus afforded rats by feeding tissues of animals killed by the same germ.

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The experiments briefly reported here were undertaken in order to learn whether the resistance of rats to pneumococcus could be increased by feeding them the tissues of animals killed by injections of the same organism. It was thought that if a toxic substance is formed in the tissues of an infected animal, and if such a poison were even only partially absorbed from the intestines of the rats to which the tissue was fed, the formation of an antitoxin might be expected. As a result, an increased resistance to injections of the living organism would *perhaps* follow. Consideration, however, of the immunity experimentally produced in such diseases as typhoid, tuberculosis and diphtheria by the oral administration of the organisms causing these diseases suggested that the pneumococci present in the tissue being fed might be partly or even wholly responsible for any protection which might be created.

A number of rats from a single source was divided into two groups, one (control) was fed the tissues of healthy rats, the other (experimental) the tissues of rats killed by intraperitoneal injections of pneumococcus Type I. Following such feedings for a period of about three weeks, with a daily average ingestion of approximately seven grams per rat, controls and experimental animals were tested. A 24 hour blood broth culture of the same germ was used.

The results of one experiment are given in the accompanying table. The data show that the experimental rats survived 1000 or more times the dose which proved fatal for control rats of equal weight. Similar results were obtained on a somewhat larger number of animals in another experiment.

The effect of feeding the living pneumococcus alone was also studied. Varying numbers of cocci were fed. Rats which received two cc. of a 24 hour blood broth culture per day for 24

days showed signs of a slightly increased resistance. Another group was given the organisms alone. Each rat received each day for 23 days the germs from 10 cc. of culture with a lapse of two weeks between the first 18 and the last 5 days of treatment. These rats also showed some signs of increased resistance. Each rat in a third group was given the germs from 18 cc. culture per day during the same period. These animals show a greater degree of protection than the ones which were given smaller amounts, although the results are not so striking as in the tissue feeding experiments. The data for these experiments will be published later. Other rats which received the bacteria from 50 cc. culture per day showed a decidedly increased resistance to injections of the germ.

Table Showing Increased Resistance to Pneumococcus of Rats Which Were Fed Pneumococcus Tissue.

Rat No.	Control or Experim'tal	Quantity Injected cc.	Result.	Date. 1925.
4	C	10^{-3}	D. 4 days	June 30
22	E	10^{-1}	Survived	
6	C	10^{-4}	D. 5 days	July 1
23	E	10^{-1}	Survived	
2	C	10^{-4}	D. 5 days	2
24	E	10^{-1}	Survived	
	stock	10^{-4}	D. 2 days	
	stock	10^{-4}	D. 4 days	
1	C	10^{-5}	D. 2 days	5
3	C	10^{-5}	D. 2 days	6
5	C	10^{-5}	D. 6 days	
18	E	$2x10^{-1}$	D. 5 days	
30	E	10^{-1}	Survived	
10	C	10^{-5}	D. 2 days	9
12	C	10^{-5}	Survived	
13	C	10^{-5}	D. 3 days	
14	C	10^{-5}	D. 3 days	
25	E	10^{-1}	Survived	
26	E	10^{-1}	Survived	
29	E	10^{-1}	Survived	
15	C	10^{-6}	Survived	14
11	C	10^{-6}	Survived	
7	C	10^{-5}	Survived	
8	C	10^{-5}	Survived	
16	E	10^{-1}	Survived	
21	E	10^{-1}	Survived	
17	E	$2x10^{-1}$	Survived	
20	E	$2x10^{-1}$	D. 2 days	

Eighteen rats, each receiving five cc. of pneumococcus culture filtrate (Berkfeld) per day for the same period, failed to show that any protection against pneumococcus had been created.

The sera of four of the rats which were fed pneumococcus tissues were tested for agglutinins, precipitins and protective substances. Although used undiluted, the sera showed neither agglutinins nor precipitins during a two hour incubation period. However, protective substances of some kind do exist. Mice, injected with 0.20 cc. of such immune rat serum at the same time as they received the pneumococcus culture are protected against many times a dose which otherwise is fatal. Control experiments with normal rat serum were done at the same time.

At present an experiment is being carried out to determine whether rats which have been fed the tissues of animals killed by pneumococcus Type 1 are protected against Types 2 and 3 as well as against Type 1.

The work is being continued from several angles. Attempts to duplicate the favorable results obtained are to be made, using larger animals. Considerably larger numbers of pneumococci, both living and dead, are now being fed. Additional work is to be done on the value of the immune serum as a prophylactic agent. The duration of the protection and the therapeutic properties of the immune serum are to be determined.

2885

The effect of dye "blockade" on anaphylaxis and antibody formation in the guinea pig.

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The experiments reported here are a continuation of the work of Gay and Clark,[1] on the effect of endothelial blockade in antibody production.

In the present work guinea pigs were saturated with trypan

[1] PROC. SOC. EXP. BIOL. AND MED., 1924, xxii, 1.

blue (daily 1 cc. injections of a 1 per cent solution of the dye in distilled water). After ten injections the animals received sheep cells, according to the method of Lewis and Loomis.[2] The trypan blue injections were then continued until the first bleeding. The results are shown in the following table. Each group represents eight to ten animals.

TABLE—Average Serum Dilution Giving Complete Hemolysis.
Bleedings (No. Days after Sheep Cell Injection)

	5	8	9	18	22	30	33
Group I, Controls	600				1800		
Trypan Blue	10				865*		
Group II, Controls		1230		570		195	
Trypan Blue		12		25		100	
Group III, Controls			700		600		0**
Trypan Blue			10		325		0***

*Represents the average of the sera of 3 pigs: 1:100, 1:500, 1:2000.
**1 animal.
***2 animals.

In practically every case the pigs treated with trypan blue produced less hemolysin than did the controls. These figures indicate a gradual rise in the titre in the trypan blue animals, but in four cases the blood consistently contained no hemolysin, even after the twenty-second day.

These results are in complete agreement with those published by Gay and Clark.

Experiments were next tried to see what relation trypan blue blockade bore to anaphylaxis. Sensitized guinea pigs were given several injections of trypan blue. On reinjection of antigen all responded vigorously. Normal animals, injected with trypan blue for a period of ten days, sensitized with egg white and then further treated with trypan blue for two weeks, responded anaphylactically on reinjection of antigen. Thus it is clear that endothelial blockade, at least in guinea pigs, neither acts as an antisensitizer, nor as a desensitizer.

There appears, then, to be a different mechanism involved in the production of anaphylactic sensitization and the formation of antisheep hemolysin in guinea pigs.

[2] *J. Exp. Med.*, 1924, xi, 503.

2886

Effect of electrolytes on the rate of inactivation of bacteriophage during precipitation.

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In view of the ease with which the bacteriophage principle is adsorbed, it is in the majority of instances carried down in the sediment when lytic filtrates are caused to precipitate. In certain cases the lytic agent remains active in the sediment and can be recovered by solution of the latter, and in other cases after adsorption it becomes inactive.

For instance, if lytic filtrate, as ordinarily prepared, is precipitated by an excess of acetone, the bacteriophage can be recovered from the precipitate. However, if the NaCl concentration of the filtrate is increased to 1 per cent or over, 99 per cent of the phage is lost within a short time after the addition of acetone. The inactivating effect of salts with divalent cations is even more pronounced, and in certain instances, even 0.05 molar concentration of salt produces rapid and complete inactivation of lytic agent, when acetone is added. If salts with monovalent and divalent cations are mixed in suitable proportions, they antagonize one another in producing this effect. Similar antagonistic effect of salts is observed also in the case of alcohol precipitation of the bacteriophage. Thus, if a small amount of $CaCl_2$ is added to a lytic filtrate, as ordinarily prepared, it becomes considerably less subject to injury by the alcohol, in virtue of the fact that the effect of the NaCl contained in the filtrate is diminished by the addition of $CaCl_2$.

2887

Anaphylactic shock caused by antibodies in animals treated with antigen; reversed passive anaphylaxis.

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Passive sensitization shows that the serum contains an antibody which has an essential part in the production of anaphylaxis. The presence of this antibody renders the animal sensitive to the action of the antigen and the character of the ensuing reaction is determined by functional peculiarities of various tissues of the body. If the union of antigen and antibody is sufficient to produce these changes it would be possible not only to sensitize animals to antigen by administration of antibody but to sensitize to antibody by previous treatment with antigen as well. One of us[1] has published observations which show that an animal treated with horse serum reacts with acute inflammation when serum of a rabbit immunized against horse serum is injected into its dermis. The usual procedure employed to produce the Arthus phenomenon has been reversed and inflammatory oedema is caused by the antibody injected into the skin of animals sensitized by antigen.

We have found that anaphylactic shock occurs in rabbits sensitized by previous injection of horse serum, beef serum or egg white when the corresponding anti-serum is injected into the blood stream. Guinea pigs have proven unfavorable for these experiments; on the one hand, rabbit's serum is toxic for guinea pigs in the amount required for the experiment and on the other hand antibody formation is relatively weak in guinea pigs so that guinea pig serum is ineffective.

It is noteworthy that when precipitin and its antigen are brought together, minimum precipitation occurs when the amount of anti-serum is several hundred times that of the antigen employed in its production. Likewise in the production of anaphylactic shock, the volume of antiserum required is greatly in excess of the corresponding antigen. Large rabbits were not suitable for these experiments because with them the production

[1] Opie, E. L., *J. of Immun.*, 1924, ix, 255.

of anaphylactic shock requires quantities of antiserum larger than those conveniently obtainable. Young rabbits weighing from 200 to 400 grams were used.

One half cubic centimeter of horse serum sensitized young rabbits to the action of from 8 to 10 cc. of strong anti-serum obtained from animals which reacted with necrosis to subcutaneous injections of horse serum. Larger doses of weaker sera (10 to 20 cc.) were needed to produce the same result. The precipitin content of the serum is an index of its toxicity when injected into these sensitized animals.

The symptoms of this reversed passive anaphylaxis do not differ from those obtainable after active sensitization or in animals passively sensitized by the usual procedure. With shock following injection of anti-serum there is muscular weakness and diminished activity of reflexes; respiration is often for a time slow and labored; urine and feces are passed and convulsive movements may occur. The animal recovers its strength after 10 to 15 minutes. A larger quantity of anti-serum causes violent convulsions and death usually within five minutes but occasionally complete paralysis and death are almost immediate. All experiments have been controlled by the intravenous injection of anti-serum into normal animals. Rabbits of the size selected tolerate antiserum in quantities much in excess of their blood volume, if the serum contains no solid particles and is free from antigen.

In order to produce maximum sensitization an interval must elapse between the injection of antigen and of anti-serum but even when antigen has been followed by anti-serum within thirty seconds death has occurred in one instance and shock has been noted in six of nine experiments. After 4 to 6 hours, shock, occasionally with death, has occurred in all instances, but the reaction is not as severe as that observed after an interval of 12 hours, when maximum intensity is reached. These experiments indicate that there is a period of incubation during which tissues concerned in the reaction presumably acquire increasing concentration of antibody.

Nevertheless it is noteworthy that an incubation period is not essential to the production of the reaction, for, slight shock may follow the injection of antigen and antibody mixed immediately before introduction into the vein of a normal rabbit. When mixtures of anti-serum derived from several immunized rabbits are

employed, the presence of antigen must be excluded, for in some weakly immunized animals antigen persists during long periods within the blood stream.

This reversed passive anaphylaxis suggests a simple explanation of the phenomena of anaphylaxis. Characteristic reactions occur whenever antigen and antibody meet within tissues, which are in consequence of their peculiar functions susceptible to stimulation or injury. This explanation does not exclude the possibility that changes resulting in symptoms may occur within the blood stream. An analogous series of events are observed when antigen is injected into the skin of sensitized animals; acute inflammation (Arthus phenomenon) follows. This tissue sensitization may be produced passively and, as pointed out above, the usual procedure may be reversed, acute inflammation being produced by intracutaneous injection of anti-serum into animals sensitized by antigen. In this experiment the reversible relation of antigen and antibody to the changes which occur in the tissues is more evident than in anaphylactic shock but in each instance antigen and antibody has caused a reaction, the character of which is determined by functional peculiarities of the affected tissue.

2888

Studies in adrenal insufficiency.

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Duration of survival of pregnant dogs after adrenalectomy.— The period of survival has been seen to be greatly increased in pregnancy. Two examples will be given: one in which the second adrenal was removed soon (probably no more than a week) after impregnation, and another in which gestation was from half to two-thirds over when the second adrenalectomy was performed. One dog (1036), known to have been impregnated in the interval between removal of the first and second adrenal, lived 46 days 3

hours after the second operation. Even then the immediate cause of death was perforation of a duodenal ulcer (15 to 20 mm. in diameter). The fatal symptoms developed abruptly on the 46th day. The coma was temporarily relieved by intravenous injections of Ringer-dextrose solution and 3 pups were born, two of them dead. Three more were found at autopsy, which was performed immediately. The breasts did not seem to contain milk. The pups were probably within about a week of full term. As the only coition took place 5 to 8 days before removal of the second adrenal, impregnation could not have occurred more than 54 days before delivery. The average period of gestation in dogs is in the neighborhood of 60 days. It is a matter of speculation how much the survival period would have been lengthened but for the complication of the duodenal ulcer. All that can be said is that it would certainly have exceeded 46 days. In our experience ulcers (gastric and duodenal) are not very commonly encountered *post mortem* in dogs dying from adrenal deficiency (5 times in 31 autopsies carried out immediately after death). The case mentioned is the only one out of 60 animals in which death was due to perforation. It is evident that the survival period was far greater than in control animals (males or non-pregnant females), 7 times greater than the average. In fact the animal lived considerably longer than any of those referred to in a previous paper,[1] whose lives had been prolonged by regular injections of Ringer-dextrose solution.

In another bitch (1034), the second adrenal was removed 24 days before the birth of 5 pups, apparently at full term. Impregnation must have occurred about 4 weeks before removal of the first, and 5 weeks before removal of the second adrenal. In other words, when the acute adrenal insufficiency was produced in the mother the embryos had completed nearly two-thirds of their intra-uterine life. She remained in good health till the pups were born and for about 3 days thereafter. Lactation was normal and the pups were well nursed. At the end of this time, however, she was found in coma and died soon after. Lengthened survival has been seen under conditions which preclude any influence of the adrenals of the embryos. For instance, in a bitch parturition began one-half hour after removal of the second adrenal. She sur-

[1] Stewart, G. N., and Rogoff, J. M., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 394.

vived 26 days, nursing 3 pups excellently. This is suggestive of a possible life-prolonging influence due to changed metabolism in the pregnant and lactating mother or to some more specific effect of certain maternal organs (uterus, *corpus luteum,* interstitial cells of ovary,?). Obviously pregnant animals must henceforth be excluded as controls.

Duration of survival of control animals.—Of 25 male control dogs, 2 survived the removal of the second adrenal for 2 1/2 days; 2 for 3 1/2 days; 1 for 4 days, 3 hours; 2 for 4 3/4 days; 1 for 5 days, 2 hours; 2 for 5 1/2 days; 2 for 5 3/4 days; 1 for 6 days, 7 hours; 1 for 6 2/3 days; 1 for 7 days; 1 for 7 days, 3 hours; 1 for 7 days, 4 hours; 1 for 7 3/4 days; 1 for 8 days; 1 for 8 1/2 days; 1 for 9 days; 3 for 9 1/2 days; and 1 for 10 days. Average 6.4 days.

Of 16 non-pregnant females, one lived after removal of the second adrenal for 2 days, 7 hours; 1 for 3 days, 10 hours; 1 for 3 1/2 days; 1 for 4 1/2 days; 1 for 4 2/3 days; 1 for 5 days, 4 hours; 1 for 5 1/2 days; 1 for 7 days; 1 for 7 days, 5 hours; 1 for 7 days, 8 hours; 1 for 7 1/2 days; 1 for 8 3/4 days; 1 for 9 days, 4 hours; 1 for 9 1/2 days; 1 for 10 days; 1 for 10 days, 9 hours. Average 6.6 days.

Autopsy findings.—While the hemorrhagic congestion in the gastro-intestinal mucosa, with blood in the lumen, is very common in greater or less degree, there are animals, whose death cannot be attributed to anything else than adrenal deficiency, in which one or both of these conditions may be absent. Thus, in 5 out of 52 adrenalectomized dogs (about 10 per cent) there was no congestion or hemorrhage of any part of the mucosa, or at least nothing more than may sometimes be seen in dogs not deprived of their adrenals and not known to be suffering from any disease. Adopting a necessarily arbitrary scale, with + signs to indicate the severity of the changes, we classify somewhat less than half of the adrenalectomized animals as showing severe and extensive congestion of the mucosa, often from stomach to rectum. In a second group, comprising about one-fifth of the cases, the condition was well marked, though less severe and less extensive. In a third group, comprising about a quarter of the cases, the condition was less marked, although recognizable, and generally it was more localized. Microscopically it is seen that the distribution of the congestion varies also in depth in the different

cases, sometimes involving the whole depth of the mucosa, in other cases only a zone next the lumen.

Congestion of the pancreas is even more common than congestion of the gastro-intestinal mucosa. In only 2, possibly 3, cases out of 53 has it been missed and in the great majority of cases it is well marked as compared with normal animals. Microscopically the islets have been found well injected but the greatest distension is in the veins in the tuberculæ. In 20 control dogs used for various purposes but not adrenalectomized, and killed with chloroform, or in other ways, only twice was a mild degree of congestion of the pancreas observed.

Presence of blood or blood pigment in the gastro-intestinal contents.—The amount of blood varied greatly but was often very considerable. In the great majority of cases it was mixed with more or less bile. Frequently blood clots were present on the mucosa. Blood pigment in the contents was often in the form of hematin. Only in 4 adrenalectomized dogs, out of 54, was no blood or blood pigment found. In 3 of these animals no congestion and no hemorrhagic condition were noted in any part of the gastro-intestinal mucosa; in the remaining dog, congestion was noted, but it was mild and not extensive. Animals dying of adrenal deficiency do not eat for a while before death, and there is no reason to suppose that blood pigment found in the gut could have come from the food.

Blood examinations.—In 20 of the dogs blood examinations were made, including estimation of sugar (Folin-Wu), of hemoglobin (comparative, with Haldane's standard); erythrocyte and leucocyte counts; conductivity of blood and serum with calculation of the number of cc. of serum in 100 cc. of blood; and often sp. gr. of blood and serum. Specimens of the results are given in the table. Dogs 1035 and 1037 were males, the rest females. It was common to find towards the end of the survival period, perhaps only on the day before death or the day of death, that the relative volume of serum dropped sharply. This was not infrequently accompanied by a diminution in the conductivity of the serum. The conductivity of serum tends to remain so constant under ordinary conditions that more weight can be given to relatively small variations. Occasionally we have thought that "concentration", with a concomitant diminution in the conductivity of the serum, could be observed beginning in the period of good

Dog	Date	Blood Sugar	Hb	Erythrocytes	K x 104 at 25° C. Blood	K x 104 at 25° C. Serum	Serum Per cent	Remarks.
1020	May 13	0.10						
	May 14			6,320,000				2nd adrenal out after blood got.
	May 16	0.095		6,240,000	58.8	126.2	63.9	
	May 18	0.077	90	6,440,000	53.7	120.0	62.4	
	May 20	0.085						
	May 23	0.074	90	6,250,000	52.5	119.5	61.0	Sp. gr. 1,058. Blood got day before death.
1025	June 4	0.10	100	7,740,000	55.3	132.0	58.8	2nd adrenal out June 5.
	June 8	0.108	99	7,100,000	43.1	127.2	49.6	
	June 10	0.105	120	9,100,000	30.3	120.0	38.6	
	June 12	0.118	122	8,500,000	30.6	120.0	38.1	
	June 14	0.083	128	9,100,000	26.8	117.8	35.2	Serum sp. gr. 1,026. Dead in night.
1028	May 21			6,550,000				
	May 22	0.108	94	6,250,000				Sp. gr. 1,056. 2nd adrenal out after blood got.
	May 25	0.082	116	8,100,000	34.4	115.0	44.7	
	May 26	0.095	96	7,700,000	38.7	115.5	49.0	
	May 28	0.093	114	8,500,000	31.0	115.0	40.9	
	May 29	0.069	120	9,350,000	25.9	108.8	36.7	Died in night.
1034	May 28	0.087	94	6,450,000	55.5	131.7	59.2	
	June 1	0.093	88	6,000,000				1st adrenal out after blood got.
	June 8	0.094	74	5,480,000	64.2	127.8	67.3	2nd adrenal out June 9.
	June 13	0.121	84	6,200,000	68.0	125.6	71.2	
	June 19	0.118	66	4,200,000	76.0	129.6	74.9	
	June 23	0.045	66	4,700,000	70.8	142.4	66.9	
	June 26	0.11	50	3,950,000	75.3	133.8	77.5	5 pups born July 2 to 3.
	July 7	0.051	102	12,000,000	37.7	128.4	45.3	Blood got just before death.
1035	May 28	0.09	98	5,600,000	54.2	132.4	57.7	
	June 1	0.082	92	5,700,000				1st adrenal out after blood got.
	June 8	0.10	82	6,400,000	57.9	124.9	63.5	2nd adrenal out June 9. Has "snuffles."
	June 10	0.066	92	6,000,000	45.6	120.9	54.1	Died during the night.
1036	June 2	0.088	88	5,900,000	47.6	130.6	52.7	1st adrenal out June 3.
	June 11	0.074	104	8,500,000	41.6	128.9	47.7	2nd adrenal out June 12.
	June 15	0.08	110	7,250,000	35.4	124.3	42.8	Sp. gr. blood 1,0697; serum 1,0246.
	June 19	0.91	102	6,200,000	39.5	123.7	47.2	
	June 23	0.08	102	7,850,000	45.3	142.4	47.1	
	June 26	0.09	102	8,500,000	43.5	133.8	47.9	
	July 9	0.093	100	8,500,000	38.2	131.7	43.5	Died July 28.
1037	June 2	0.084	110	7,800,000	41.6	128.8	47.6	1st adrenal out June 3.
	June 11	0.091	108	8,800,000	42.1	128.2	48.3	2nd adrenal out June 12.
	June 15	0.085	118	8,000,000	34.1	124.9	41.3	Sp. gr. blood 1,0644; serum 1,0239.
	June 19	0.074	126	9,700,000	23.2		29.4	Died night June 21 to 22.
1038	June 2	0.09	108	7,200,000	46.5	132.4	51.1	1st adrenal out June 3.
	June 10	0.09	106	6,400,000	45.9	132.5	50.6	2nd adrenal out after blood got.
	June 13	0.091	130	10,200,000	30.5	123.7	37.9	
	June 16	0.085	116	8,200,000	25.7	116.0	34.4	Sp. gr. blood 1,0702; serum 1,0252.
	June 18	0.058	128	8,500,000	21.3	116.6	29.1	Sp. gr. blood 1,071; serum 1,0233.
	June 19	0.07	122	8,250,000	17.1		23.7	Died 2 hours after blood got.

health before the animal began to refuse food, and presaging the fatal change. But too much stress ought not to be laid on this or on the terminal "concentration" of the blood. The latter is seen in dog 1035, which is for this reason included in the table, although in all probability it did not die of adrenal deficiency. In dog 1034, instead of a concentration, there is some dilution of the blood after removal of the second adrenal, although the terminal concentration is seen. In this animal during the period of good health there was one apparent relapse, when the blood sugar sank to 0.045 per cent., and it looked as if the animal was going to die. She recovered, however, and lived a fortnight longer.

2889

Starch grains of wheat considered as partially dehydrated amylose.

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Alsberg and Perry[1] have shown that about 60 per cent of the starch grains is soluble in cold water if it is ground for several days in a pebble mill. This fact will be used in this paper as a basis for an explanation of the properties and structure of wheat starch grains. The assumption that amylose occurs in starch grains in different stages of dehydration proved to be the most satisfactory working hypothesis. We will assume the grains to be built up of alternate layers of more hydrated amylose (less refractive rings) and of less hydrated amylose (refractive rings, to which belongs the surface ring). If we follow the terminology of Meyer, without accepting his theory in detail, the former, β-amylose, is not only soluble in hot water, but also in cold water; the latter, α-amylose, is not soluble in boiling water at 100° C. Of the α-amylose rings, the surface ring is the denser and the more dehydrated. It has a low swelling capacity at room temperature. Though the inner layers, being less dehydrated, tend to have a higher water content and to elongate their circumfer-

[1] Alsberg, C. L., and Perry, E. E., PROC. SOC. EXP. BIOL. AND MED., 1924, xxii, 60-61.

ence, the surface ring does not allow this. In this way, there exists an equilibrium between the swelling capacity of the inner layers and the cohesion (elasticity) of the surface layer. If the cohesion of this border layer is decreased or destroyed (by damaging), the equilibrium is broken and more water can be taken in. If this cohesion is decreased in additional α-amylose rings, greater swelling occurs, and β-amylose leaches out; if the cohesion is destroyed in still more rings (by damaging), all the β-amylose dissolves and diffuses out. The last phenomenon is caused by grinding, by which all the α-amylose rings are crushed and their continuity broken, so that the β-amylose, or 60 per cent of the total starch grains, goes into solution in the surrounding water. If these grains show places where the surface is less refractive (clear in light field and non-luminous if direct light is shut off) they are swollen unilaterally or locally. They are the first to swell if they are hydrated (by heating or by KOH) and the clear places are the first to become deeply colored in a weak I-KI solution. By heating above ±50° C. the hydration of the refractive rings (including the surface ring) increases; they are no longer continuous, but are broken locally into small granules, so that swelling occurs. After heating for a short time at 100° C. the surface ring forms a bag around the liquid β-amylose which is in solution in the interior. Treatment with tannin produces a precipitate on the outside and inside of the bag; α-amylose and β-amylose pass gradually without sharp transition point one into the other. There is only a quantitative difference in hydration between them. If the dehydration passes beyond a certain limit, agglomeration occurs, so that the α-amylose no longer disperses in water. The greater the degree of dehydration, the more promptly must we hydrate to reconvert it into β-amylose. We can then increase hydration of amylose by:

(1) Heating in water. The higher the temperature, the more hydration.
(2) KI, $Ca(NO_3)_2$, $ZnCl_2$.
(3) KOH, Na_2CO_3.
(4) Diluting with water.

We can decrease dehydration of amylose by:

(1) Lowering the temperature.
(2) Alcohol, chloroform.
(3) Tannin.

(4) Withdrawing water and concentrating the solution.

After having increased hydration in one of the four ways, we can use one of the four ways of decreasing hydration, and vice versa.

In this way, retrogradation and its different rate under different conditions can be explained.

The surface ring is always the most dehydrated part of the starch grain in agreement with the Gibbs theorem, by which the substance with the lowest surface tension is accumulated in the surface.

Crystals, in forms of needles or bars, were observed, not only when a concentrated solution of amylose was heated to 100° C., but also when an amylose solution was allowed to dry out at room temperature.

Iodine-potassium-iodide colors starch more readily and more purely blue, the more the amylose is hydrated. All shades between red and blue-black can be observed. Attention is called to the fact that potassium iodide itself has a strong hydrating power. A connection between its hydrating power and its coloring power is suggested.

2890

Transmission of dengue fever by mosquitoes.

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[*From the U. S. Army Medical Department Research Board, Manila. P. I.*]

This summarizes the results obtained in an extensive series of experiments relating to the transmission of dengue fever by mosquitoes. The investigations have been pursued by the U. S. Army Medical Department Research Board at the Bureau of Science and at the Sternberg General Hospital in Manila, P. I.

Part I of the report considers the plans and arrangements made and the preliminary work done in preparation for the actual experimental work. Part II concerns itself with the various sets of experiments made, the results obtained and the conclusions drawn therefrom.

PART I.

The preliminary arrangements had for their basis the scope of the work contemplated which covered the following points: confirmation of the reported transmission of dengue by the *Ædes (Stegomyia) egypti;* incrimination or elimination of the *C. quinquefasciatus* as a transmitter; and investigation of the exact mechanism of transmission by mosquitoes.

Only two species of mosquitoes were used in the transmission experiments—*A. egypti* and *C. quinquefasciatus* and the reasons for so doing are explained. The arguments presented indicate that no species other than these two could be concerned in the transmission of dengue in Manila.

All the mosquitoes used in the experiments were bred from the egg. A period of approximately four months was spent in testing various food substances that might be suitable for the propagation of larvæ and in developing and perfecting a routine breeding technique. Normal horse serum was finally selected for this purpose and formalin (1:2,500 to 1:5,000) was added to inhibit bacterial growth. In the later stages of the experimental work a considerable number of experiments were made in breeding larvæ in solution of tap water to which slices of ripe banana had been added and this type of food was found to be superior to blood serum. In work of a similar nature the use of banana as food for larvæ is recommended for both *A. egypti* and *C. quinquefasciatus.*

All reserve stocks of adult mosquitoes were fed on aqueous solutions of sugar and this type of food proved to be satisfactory in all respects.

As a rule the *A. egypti* were used experimentally for initial biting from two to seven days after emergence, and the *C. quinquefasciatus,* when first fed on blood, were not more than five days old.

The exact number of mosquitoes as well as the species used in each experiment was always known and the biting of human beings was always under their control and exclusively at the will of the Board.

After the infecting exposure to dengue patients, of freshly-bred mosquitoes, all not showing complete distension with blood were removed and killed. It was known, therefore, that all mosquitoes used subsequently to determine their infectivity for volunteers had been potentially infected.

Freshly-bred *Æges egypti* would not bite freely on the day of their emergence but after they had taken food (solution of sugar) and had been fertilized, they took blood freely—usually 100 per cent of them.

A. egypti in the Philippine Islands bites freely at any time during the day, and night-biting, though unusual, also is observed. The biting habits of *C. quinquefasciatus* were found to be erratic; they would take blood only at night, and even under the most favorable conditions but a relatively small proportion would do so.

The experimental subjects consisted of military personnel that proffered their services voluntarily. Sixty-four men were used. The volunteers were specially selected and in general met certain basic requirements—freedom from disease, including syphilis; short service in the Philippines; and non-immunity to dengue.

The experiments were made in a specially prepared ward in a large military hospital in Manila and extraordinary precautions were taken to exclude mosquitoes. The ward was administered by specially selected personnel, and one of their most important functions was the detection and destruction of mosquitoes that might possibly gain entrance to the ward or its vestibules.

PART II.

The transmission experiments presented in this report include a total of fourteen injections of virus blood, of which five were positive, one hundred and eleven biting experiments with *A. egypti* of which forty-seven were positive, and seven biting experiments with *C. quinquefasciatus*, all of which gave negative results. Sixty-four volunteers were used and dengue was produced experimentally in fifty-two instances (81 per cent).

In the conduct of the experiments, the general policy was adopted, in each series, of using successively all available methods for producing the disease—biting, followed in many instances by repetition, and this followed in turn by injections of virus blood. All negative results were adequately controlled—biting by mosquitoes known to be infective or by injections of virus blood.

Previous reports of the transmission of dengue by the *A. egypti* were confirmed—forty-seven positive results.

Experiments were made with eight volunteers to fix the incubation period of the virus in the mosquito, and it was found that the mosquito did not become infective until the eleventh day after

its infective meal. The evidence obtained indicates that even on the eleventh day after their infection *Ædes egypti* may not be capable of transmitting the virus. The limits of incubation of the virus in the mosquito apparently are from the eleventh to the fourteenth day.

Experiments were made with twenty-one volunteers to determine the stages during which dengue patients are infective to *A. egypti*. The results obtained indicate that the patient is infective to mosquitoes during the first three days of the disease but that on the third day of an attack the mosquito frequently will fail to pick up the virus. It is demonstrated, furthermore, that individuals in the late prodromal stages of dengue—six to eighteen hours prior to onset—are infective to *Aedes*.

The experimental evidence obtained warrants the statement that once the *A. egypti* becomes capable of transmitting the virus to human beings this characteristic is retained throughout the remainder of the mosquito's life. Experimental dengue was produced in three volunteers with mosquitoes that had been infected respectively sixty-two, sixty-six and seventy-five days previously.

Endeavors were made to infect seven volunteers with potentially infected *C. quinquefasciatus (C. fatigans)* and all such experiments were entirely negative. The volunteers were then bitten by *A. egypti* infected from the same sources and on the same day as the *C. quinquefasciatus* used in the previous experiments and all came down with dengue.

The conclusion is drawn that *C. quinquefasciatus* does not transmit dengue.

Three volunteers were used to ascertain the possibility of the hereditary transmission of the virus in the mosquito. The results obtained were entirely negative. When the experimental subjects were subjected to control experiments, all three developed dengue. The evidence suggests very definitely that the virus of dengue fever is not carried from the infected *A. egypti* through its eggs to the next succeeding generation.

The incubation period of the disease in the forty-seven experimental cases varied from four to ten days inclusive. For all practical purposes the incubation period may be considered as being from four to six days inclusive, as it fell within that period in 89 per cent of the experimental cases reported.

In forty-one of the forty-seven cases of dengue experimentally

produced, the virus was derived from the same strain and this strain was passed from man to mosquito and back to man through six generations. There was no evidence that the virus suffered attenuation nor that its virulence was increased as a result of continuous alternate passage through man and mosquito.

The numbers of potentially infected *Aedes* that took blood for infecting purposes in the forty-seven positive cases varied from two to thirty-six, and 50 per cent of the positive cases were bitten by from two to ten potentially infected mosquitoes.

The preliminary periods of isolation and time interval intervening between biting experiments, with one exception, was not less than eight days and did not exceed eighteen days.

2891

Studies on the biology of the streptococcus erysipelatis. IV. Toxin production of the streptococcus erysipelatis.

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Studies of toxin production by *Streptococcus erysipelatis,* and on neutralization of this toxin by the serum of convalescent erysipelas patients, and by erysipelas antistreptococcic rabbit and donkey sera, which were begun by me in the Medical Clinic at the Johns Hopkins Hospital in the fall of 1924, and continued in this laboratory, add further evidence to my previous reports[1] that a specific relationship exists between *Streptococcus erysipelatis* and erysipelas. The toxins employed in these studies were prepared in 48 hours' tryptic broth medium cultures of *Streptococcus erysipelatis,* incubated at 37° C. Thirty-four strains tested were found to yield uniformly toxic filtrates. The tryptic medium employed was the original Douglas'[2] tryptic medium digest, modified by Hartley,[3] Watson and Wallace.[4] From a large

[1] Birkhaug, K. E., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 292; *Bull. Johns Hopkins Hosp.,* 1925, xxxvi, 248; *ibid.,* 1925, xxxvii, 85; *ibid.,* 1925, xxxvii, 307.

[2] Douglas, S. R., *Lancet,* 1914, ii, 891.

[3] Hartley, P., *J. Path. and Bact.,* 1922, xxv, 479.

[4] Watson, A. F., and Wallace, U., *J. Path. and Bact.,* 1923, xxvi, 447.

series of toxic filtrates incubated over periods varying from six to ninety-six hours, the greatest toxin production was found to occur about 48 hours after inoculation of the medium. A definite decrease in toxin concentration took place about 90 hours after the initial incubation. The curve of toxin production and concentration was determined in the skin of persons susceptible to a skin test dose of 0.1 cc. of a 1:1000 dilution in normal saline solution of the toxic filtrate. The first appearance of sufficient toxin concentration to render a positive skin lesion, measuring more than 5 mm. in diameter, occurred about 12 hours after the inoculation of the medium, and the lesion produced by the toxic filtrate incubated for 24 hours, measured about 1.5 cm. in diameter. The lesion produced by one skin test dose of the toxic filtrates from the cultures incubated for periods of 48, 96, and 120 hours, measured respectively 3.6 cm., 3 cm., and 2.4 cm., in diameter. The lesions were similar to those observed in the Dick test for scarlet fever susceptibility. The skin lesions were read 24 hours after the injection of the skin test dose. The lesion rapidly disappeared about 60 hours after the injection of the skin test dose, and only occasionally was found to leave behind a slightly pigmented area. Complete neutralization of the toxin was obtained by mixing the skin test dose with an equal amount of convalescent erysipelas serum, or with 0.001 cc. of erysipelas antistreptococcic rabbit or donkey sera.

Thermal inactivation of the toxic filtrates was first detected after heating the filtrates at 90° C. for one hour. The lethal dose of the toxin for rabbits varied from 2 to 12 cc. per kilogram of weight, death occurring from 8 to 96 hours after the intravenous, intraperitoneal, or intramuscular injection of the toxic filtrate. Rabbits susceptible by the skin test to one skin test dose of the toxin, succumbed rapidly following the intravenous administration of 3 to 5 cc. of the toxic filtrates. Six rabbits out of 37 animals tested, or 16 per cent, gave uniform skin lesions with one skin test dose. It was clear, however, from testing a large number of laboratory animals that such material was unsatisfactory for the titration of a standard skin test dose. Persons susceptible to the erysipelas streptococcic toxic filtrates were employed for the purpose of titration of an adequate skin test dose.

Eighteen cases of erysipelas injected with a skin test dose of the toxin gave positive lesions on their arrival at the hospital.

During defervescence of the disease and the regression of the erysipelatous lesion, the reaction to one skin test dose of the toxin became rapidly obscured. The shortest period in which a patient's positive skin reaction became negative was 5 days after the onset of the disease, and the longest period recorded was 38 days after admission to the hospital. The blood serum from erysipelas patients with positive skin reactions, when injected intradermally in normal, but susceptible individuals, gave positive skin reactions. When the patient's blood serum was mixed with an equal amount of convalescent erysipelas serum, or with 0.001 cc. of erysipelas antistreptococcic donkey or rabbit sera, the toxin was completely neutralized. During the period of infection in which the patient's blood serum contained enough toxin to produce a positive skin lesion in susceptible persons, the patient's urine contained a similar toxic substance, which was completely neutralized by convalescent erysipelas serum, or by the erysipelas antistreptococcic donkey or rabbit sera. The toxic substance in the urine was obtained by passing the fresh urine through a Berkefeld V candle and by diluting the filtrate in physiological saline solution up to 1:500. A skin test dose of 0.1 cc. of this dilution uniformly produced a positive skin lesion in the patient or susceptible persons.

Erysipelas patients whose skin reactions were positive on admission to the hospital, when treated with therapeutic doses of erysipelas antistreptococcic donkey or rabbit sera (25 to 100 cc.) gave negative skin reactions with multiple skin test doses of the toxin as soon as 12 hours after the intramuscular administration of the serum. If the disease persisted unchecked by the serum therapy, the skin reaction remained positive until defervescence and definite regression of the erysipelatous lesion occurred.

Among 135 hospital patients admitted with other complaints than erysipelas and ranging in ages from 18 to 72 years, thirty-six patients, or 27 per cent, gave positive skin reactions with one skin test dose. Among nineteen patients, with definite histories of erysipelas, from one to twelve years ago, four gave positive skin reactions.

Among 272 normal school children, ranging in ages from 7 to 17 years, fifty-seven persons, or 21 per cent, gave positive skin reactions with one skin test dose. When 251 of the same children were tested with one skin test dose of the Dick scarlet fever toxin, one hundred and thirty-one persons, or 52 per cent, gave

positive skin reactions. Only 25 of these children, or 10 per cent, gave simultaneously positive reactions when tested at once with the erysipelas streptococcic toxin and the Dick scarlet fever toxin.

Neutralization of the erysipelas toxin, as judged by the human skin test, was accomplished by convalescent erysipelas serum and the erysipelas antistreptococcic donkey or rabbit sera, and not by Dochez' scarlatinal antistreptococcic serum, nor by normal rabbit or donkey sera.

Summary.

1. Among 34 strains of *Streptococcus erysipelatis* grown in tryptic medium at 37° C., the maximum toxin concentration was obtained in the lots incubated about 48 hours. 2. A skin test dose of 0.1 cc. of a 1:1000 dilution of erysipelas toxic filtrate produced in the skin of susceptible persons a lesion which measured more than 1.5 cm. in diameter. 3. Complete neutralization of one skin test dose of the erysipelas toxin was obtained by mixing it with an equal amount of convalescent erysipelas serum, or 0.001 cc. of erysipelas antistreptococcic rabbit or donkey sera. 4. During the acute stages of erysipelas the patient's blood serum and urine contained a toxic substance which was completely neutralized by convalescent erysipelas serum and which disappeared from the patient's blood serum and urine as soon as twelve hours after the administration intramuscularly of 25 to 100 cc. of the erysipelas antistreptococcic rabbit or donkey sera. 5. Positive skin reactions were obtained by one skin test dose of erysipelas streptococcic toxin in 27 per cent of apparently normal adults and 21 per cent of normal school children. 6. Among nineteen persons with definite histories of single and recurrent attacks of erysipelas, 4 persons gave positive reactions with one skin test dose of the erysipelas streptococcic toxin. 7. Neutralization of the erysipelas streptococcic toxin was not accomplished by Dochez' scarlatinal antistreptococcic serum, nor by normal rabbit or horse sera.

2892

Blood clot method of immunization with observations on pneumococcus toxemia.

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Recent work in scarlet fever and the staphylococcus toxin work of J. T. Parker[1] have obviously suggested renewed inquiry into pneumococcus infections by similar methods. One of us discussed this in a paper of last June.[2] The thought has surely come to many bacteriologists. Moreover, it has been tried many times before; by the Klemperers,[3] by Mosny,[4] by Tizzoni and Panichi,[5] and, particularly, by Wadsworth,[6] who discussed the differences between the antitoxic effects of sera produced with culture filtrates and the antibacterial action of those produced with the whole bacteria. Thomas and Frederick Parker,[7] furthermore, pointed out microscopic necrosis in various organs not associated with the local presence of pneumococcus.

Savchenko's[8] papers of 1905 and the Dick studies show that a bacterial substance, poisonous for man and antigenic, may be relatively harmless for all the ordinary laboratory animals; and from J. T. Parker's work we learn that the toxicity and antigenic effectiveness of some poisons may be limited to individual tissues. We are therefore approaching the problem indirectly by immunizing horses with pneumococcus preparations in the hope that eventually the serum of these horses may display antitoxic potency rather than purely antibacterial effects in clinical test. In

1 Parker, J. T., *J. Exp. Med.*, 1924, xl, 761.

2 Zinsser, Shattuck Lecture, *Boston Med. and Surg. J.*, 1925, cxcii,1191.

3 Klemperers, *Berl. Kl. Woch.*, 1891, xxviii, 833 and 869.

4 Mosny, cited from Wadsworth loc. cit., *Arch. di med. Exp.*, etc., 1892, iv, 195.

5 Tizzoni and Panichi, *Centralbl. f. Bakt.*, Ref., 1905, xxxvi, 25.

6 Wadsworth, *J. Exp. Med.*, 1912, xvi, 54 and 78.

7 Thomas and Frederick Parker, Jr., *Arch. Int. Med.*, 1920, xxvi, 125 and 132.

8 Savchenko, *Russki Vratch.*, 1905, xxv, 797; cited from Park, *J. Am. Med. Assn.*, 1925, lxxxv, 1180.

the course of this work we have developed a method which we think may be useful not only for our purposes, but for the production of sera in the other diseases mentioned. We are publishing the method now, since we think it sufficiently promising to make general trial desirable. Incidentally, we believe it has furnished us further evidence that a toxic factor develops when pneumococci grow in the body under conditions analogous to those prevailing in pneumonia. Whether or not this toxic factor is antigenic remains to be seen.

We began by treating two horses with increasing amounts of 5 day blood broth culture filtrates of Type I pneumococcus. Resulting local reactions were rarely more than slight, and doses of 500 cc. could be given within 6 weeks after beginning treatment, occasionally producing slight temperature and transient local edema. In one horse, such filtrates were alternated with a modified Dochez method consisting in the injection of 20 to 30 cc. of agar inoculated in the syringe with virulent pneumococci. The agar injections resulted in violent local and sometimes systemic reactions, with eventual abscesses. In the other horse, filtrate injections were alternated with increasing intracutaneous doses of virulent pneumococci. After three months, as much as 50 cc. blood broth cultures of the organisms were so administered with astonishingly mild reactions. This mildness is of importance in its contrast with the severe results of the agar injections, and the very severe ones obtained by the new method. It is carried out as follows:

Normal horse blood is taken into potassium oxalate solution to prevent clotting. It is inoculated with pneumococci and incubated for about 24 hours. At this time a sufficient amount of calcium chloride is added and the mixture subcutaneously injected into the horse. A subcutaneous hematoma results in which the organisms continue to multiply. In early injections the oxalated blood is not incubated with pneumococci but inoculated just before injection. In such cases, not only calcium chloride but a little horse serum, to supply thrombin, is added. Grinnell has found that incubation of oxalated horse blood with pneumococci hastens coagulation, an observation which is being further studied by him.

The injection of 40 cc. of such an infected clot in the horse which had previously shown slight reactions only to intracuta-

neous doses of 50 cc. of virulent culture resulted in a violent local reaction with an edematous swelling, spreading, hot and tender, rise of temperature to 39.4, depression and failure to feed, this subsiding gradually in about 5 days. It is also interesting that the other horse, which had had previous agar treatments, reacted less severely to a similar injection, which we hope may indicate a developing immunity.

In order to determine in a preliminary way what it is that is probably going on in the injected blood clot, pneumococci were cultivated *in vitro* in human blood clots and with suitable controls of uninoculated blood clot extracts; a number of individuals were intracutaneously tested.

Susceptibility to these materials varies very much in individuals, which is encouraging in rendering less probable a non-specific toxicity attributable to cleavage of the protein. In susceptible individuals, such as one of the writers, 1/20 of a 1:10 dilution, representing in final calculation 1/400 cc. of the original culture material, gave rise to a painful, red and swollen area, reaching its maximum in about 36 hours, at that time having a red areola over an inch in diameter, hot and tender, with some pain up and down the arm; not completely fading for 2 weeks. Another individual developed a somewhat smaller but similar reaction with the same amount, and with a negative control, while others, with half this amount, namely, 1/800 cc. of the original material, had, in recent tests, three of them definite but slight reactions with negative controls, while one individual in this group was absolutely insusceptible.

We make no particular claims at the present time either as to the specificity of this poison or its antigenic properties. We believe, however, that it represents the substance which gives a severe reaction in the horses inoculated by the blood clot method, and that its potency, relatively low as it is as compared with other bacterial poisons, is still sufficient in susceptible individuals to more than account for the clinically observed toxemia in pneumonia. Our primary purpose in presenting these facts is to submit a method which, in its ease of performance and failure to cause abscesses, the production of localized bacterial foci without foreign substances and complete eventual absorption, should be freely available for the experimental study of serum production.

The observations herein reported were made possible by the

cooperation and, indeed, collaboration of our associate, Dr. Benjamin White, who is continuing to take an active part in the further studies of the pneumococcus serum and is beginning to apply the method to scarlet fever serum production. In collaboration with Dr. White, we are further attempting the effects of using this method with some other bacteria.

2893

Effect of menotoxin injections on behavior of rats in the maze.

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Macht and Lubin[1] have described their studies on menotoxin first in these Proceedings and later in a fuller communication elsewhere.[2] It was shown that in the blood, sweat, saliva, milk and other secretions of menstruating women there is present a toxin, which is especially deleterious to plant protoplasm, but is also to a lesser degree toxic for animals and animal tissues. The chemical nature of this toxin was found by them to bear a relationship to oxycholesterin and allied bodies such as cholic acid.

It is well known that at the time of catamenia, the female organism undergoes profound metabolic and other physiological changes. Very common concomitants of menstruation are pain, malaise, nervous irritability, and psychic disturbances. In the present investigation an inquiry was made as to whether such symptoms may not be referred to the presence of menstrual toxins. Albino rats were trained to run in the circular maze, so as to perform that exercise in the shortest period of time and without errors. The rats were then injected with normal human blood serum on the one hand, and with blood serum from menstruating women, on the other hand, and the effect of the injections was observed. In order to avoid anaphylactic phenomena

[1] Macht, D. I., and Lubin, D., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 265.

[2] Macht, D. I., and Lubin, D., *J. Pharm. and Exp. Therap.*, 1924, xxii, 413.

the injections were made in different rats, and when the same rats were used again, it was only after a long period of rest. Fifteen experiments were made with injections of normal human serum, and twenty-one with menstrual serum, the doses injected ranging from 0.01 cc. to 0.2 cc.

It was found that injections of normal serum produced no effect, or occasionally a slight transient depression. Injections of menstrual serum produced very marked depression of the animals, as manifested by their speed of running, loss of orientation, and numerous errors and frequently there was a distinct paresis of the hind legs. Recovery almost invariably followed in a day or two. Injections with alcoholic extracts of normal blood serum (evaporated and taken up in saline) and of menstrual serum gave exactly similar results as above. The results obtained in the rats were somewhat analogous to those obtained by the authors with cholic acid and cholesterin derivatives which are closely related to menstrual toxin.[3]

2894

A study of the toxin of pernicious anemia.

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In a paper on the toxin of menstruation, Macht and Lubin[1] have shown that by means of phyto-pharmacological preparations, the presence of a poison or toxin in the blood menstruating individuals can be detected. While examining specimens of blood from normal and menstruating women the author happened to test a specimen of blood from a case of pernicious anemia and found it to be highly toxic for plants. The toxicity was even greater than the most toxic specimens of menstrual blood. Inasmuch as the above specimen came from a man it was thought worth while to study other cases of pernicious anemia by the

[3] Macht, D. I., and Hyndman, O., *J. Pharm. and Exp. Therap.*, 1924, xxii, 467.

[1] Macht, D. I., and Lubin, D., *J. Pharm. and Exp. Therap.*, 1924, xxii, 413.

phyto-pharmacological method. A larger series of cases of normal blood and blood from various anemias, as diagnosed clinically and morphologically, were examined and it was found that the blood serum from cases of pernicious anemia behaved differently from that of all the other cases examined.

The tests were made on living seedlings of *Lupinus albus*, described by the author elsewhere. It was found that whereas the index of growth given by a one per cent solution of normal human blood was 75 per cent, the average growth coefficient given by 48 cases of pernicious anemia was 44 per cent; some of the specimens giving as low a figure as 28 per cent and none of them giving a higher figure than 51 per cent. Specimens of severe secondary anemias and specimens obtained from patients suffering with carcinoma, pellagra, lymphatic and myologenous leukemias, and Hodgkin's disease were all found to be but little toxic for the plants so that the author had no difficulty in diagnosing cases of pernicious anemia by examining unknown samples of blood by the phyto-pharmacological method. These observations seem to speak in favor of a toxic etiology for pernicious anemia. They are also considered by the author of considerable value in making a differential diagnosis of pernicious anemia in doubtful cases; and furthermore they should prove useful to investigators in the search for the causative agent of the disease and its treatment. Fuller details will be published in the *Festschrift* of Professor Abel.

2895

Penetration of ultra violet rays through animal tissues.

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Studies on the penetration of ultra violet rays for tissues were made on living animals with the quartz spectroscope and spectrograph. For this purpose rabbits, cats and dogs were used. The animals were anesthetized, the skin of the abdomen was cut open and dissected back on one side and then the barrel of the

spectrograph was introduced under the skin. The skin was then radiated from the outside with Krohmayer and Hanovia Alpine Sun Mercury Vapor Lamps and a spectrophotograph of the waves which passed through the skin was thus obtained while the animal was alive and the blood circulating. In this way it was found that a considerable number of invisible ultra violet rays passed through the rabbits', cats' and dogs' skins. Thus in the case of rabbit skins varying from 1 to 2 or more millimeters in thickness, the spectrograph showed the lines in the region of 2800 angstrom units and sometimes even shorter wave lengths. In the case of the rabbit the longer ultra violet rays penetrated not only through the skin but even through the whole thickness of the abdominal wall as shown by spectrographs made with the barrel of the instrument inserted into the peritoneal cavity. Here wave lengths of 3000 angstrom units were frequently obtained with both Alpine Sun and Kromayer lamps, although the thickness of the abdominal wall (skin, fascia, muscle, and peritoneum) was usually about 3 to 4 millimeters.

The permeability of dead tissue was different from living animal tissue, depending upon the state of preservation. Thus, when skin was left at room temperature and began to undergo putrefaction, the permeability was greater than in a normal living skin. On the other hand when skin was preserved either by freezing or by preservatives such as formalin or alcohol, the coagulation of proteins and other chemical changes thus produced rendered it more opaque. Leather for the same reason was found to be much more opaque than living skin. Human skin was studied by obtaining fresh specimens from the operating room, and it was found that when not excessively thick it also allowed the penetration through it of the longer ultra violet waves. A marked difference was noted between white human skin and the skin of the negro. In the latter case absorption of the entire ultra violet region was noted. In rabbits and other animals under anesthetic, injections intravenously into the living circulation of fluorescent substances, such as eosin, produced complete absorption of the ultra violet rays. The present investigation, which was begun in the fall of 1923, reveals conclusively that the penetration of ultra violet rays from powerful modern quartz lamps through animal tissues is much deeper than has hitherto been supposed.

2896

Sudden death of experimental animals following intrapericardial injections of tincture of iodine.

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During the course of some experiments on rabbits, it was noted that injection of tincture of iodine (U. S. P.) into the pericardial sac was followed by some unidentified disturbance of cardiac mechanism which resulted in the death of the animals within a few minutes. The same series of events followed in each of the five animals employed. Six dogs were then studied. Under ether intratracheal anesthesia, the chest was opened and from 1.5 to 2 cc. of tincture of iodine injected, with much the same results as with the rabbits. As soon as the first few drops of the solution touched the epicardium there was an obvious visible effect on the heart muscle which continued until the death of the animal within five to ten minutes. Examining the heart grossly after death, it was found that the iodine had diffused over the entire epicardium but there was no visible evidence of staining of the heart muscle beneath the serous covering. In order to determine, if possible, the sequence of events following the injection of the irritant, the next two experiments were carried out in the Department of Physiology with the animals attached to the electrocardiograph. The first animal alone succumbed to the first dose of iodine. The second animal failed to show the usual prompt response and death did not occur until three injections of iodine had been given. We have no positive explanation to give of the phenomena observed. The rapidity of the effect of the injection would make it improbable that there was any systemic disturbance; rather it would point to a purely local action of the alcohol on the heart muscle or the electrical mechanism of the organ. The electrocardiograms showed paroxysms of ventricular tachycardia, flutter and fibrillation. After the paroxysms a peculiar rhythmical rising of the T wave was noted coming off the down stroke of the R wave at higher and higher levels and then dropping back, but finally coming off per-

sistently at a higher level and rising above the peak of the R wave, the latter appearing merely as a notch. The resulting complexes were bizarre. A.-V. block finally appeared. A shifting of the position of the auricular pacemaker was noted in P waves which became negative and returned to upright positive position. In one instance the auricular electrical phenomena continued, while in another the ventricular electrical changes continued after the auricle stopped.

2897

A structural characteristic of the cardiac poisons.

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Former investigations[1] have shown that strophanthidin is unsaturated and that the double bond is situated within the lactone ring, between the β and γ carbon atoms, so that strophanthidin may be designated as a $\Delta\beta$-γ crotonic lactone. Characteristic of strophanthidin and all of its derivatives which still possess this unsaturated lactone ring is their reducing action on Tollens' solution. On the other hand, dihydrostrophanthidin and its derivatives, or isostrophanthidin, in which the double has been either hydrogenated or shifted to another position, no longer react with Tollens' reagent, or at least far more gradually than in the case of strophanthidin and its derivatives. The behavior towards Tollens' solution is thus a very characteristic test for the unsaturated lactone group of these compounds.

Results of a similar and most striking character have been recently obtained by the use of the sodium nitroprusside test. Strophanthidin and all of its derivatives which still possess the unsaturated lactone ring give positive reactions with this reagent. But as soon as this group is hydrogenated or lost by saponifica-

[1] Jacobs, W. A., and Collins, A. M., *J. Biol. Chem.*, 1925, lxiv, 383; 1925, lxv, 493.

tion to the acid, the resulting substances no longer give this reaction.

In the course of structural studies with ouabain this substance has been found to be unsaturated since it absorbed two mols. of hydrogen with the formation of a tetrahydro derivative. It contains also a lactone group. Because of our experience with strophanthidin, it was of interest to establish the fact whether the lactone group and an unsaturated linking were associated. Ouabain was found to reduce Tollens' reagent and gave also a positive nitroprusside test. On the other hand, tetrahydroouabain failed to react with these reagents. After saponification ouabain likewise no longer reacted with sodium nitroprusside. There appears, therefore, to be a very strong indication that ouabain, like strophanthidin, possesses an unsaturated lactone group.

A similar study has been made of the behavior toward these reagents of the digitalis glucosides, digitoxin, and gitoxin which contain different although possibly related aglucones, respectively digitoxigenin and gitoxigenin. The latter has been demonstrated by Windaus and Schwarte[2] to be a lactone and also to be unsaturated. Although Kiliani had shown digitoxigenin to be a lactone, his hydrogenation experiments with the substance were unsuccessful.[3] However, if one adopts Cloetta's[4] formula, $C_{24}H_{36}O_4$, and the view that it is tetracyclic, it must contain one double bond and, therefore, like gitoxigenin, it is a lactone and at the same time unsaturated. We have studied the behavior of these substances towards Tollens' reagent and sodium nitroprusside and obtained definitely positive reactions in both cases. After gentle saponification, digitoxin and gitoxin no longer gave positive reactions with sodium nitroprusside. Here again the olefinic group must be associated with the lactone group, and there is a very strong suggestion that digitoxigenin and gitoxigenin also possess unsaturated lactone groups.

Of great importance in connection with these results is the interesting observation of Windaus, Bohne and Schwieger[5] that hydrogenation of the unsaturated group of digitalin (a glucoside

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[2] Windaus, A., and Schwarte, G., *Ber. Chem. Ges.*, 1925, lviii, 1515.

[3] Kiliani, H., *Ber. Chem. Ges.*, 1918, li, 1631.

[4] Cloetta, M., *Arch. Exp. Path. Pharm.*, 1920, lxxxviii, 133.

[5] Windaus, A. Bohne, A., and Schwieger, A., *Ber. Chem. Ges.*, -924, lvii, 1386.

in all likelihood of gitoxigenin) renders the latter practically non-toxic. A few similar comparative toxicity tests which we have made with tetrahydroouabain have shown it to be at least one hundred times less toxic for frogs than ouabain.

From these observations there is a very strong suggestion that the aglucones of ouabain, the digitalis glucosides and perhaps of other substances of this pharmacological group such as bufotalin, possess, like strophanthidin, an unsaturated lactone group; and that this group may be essential, perhaps in conjunction with other structural features, for the pharmacodynamic action of these substances. We are at present attempting to ascertain by more direct chemical methods, as was accomplished in the case of strophanthidin, whether these substances are indeed inner esters of enolized ketones; and we are also attempting to substantiate by further work the suggested pharmacodynamic significance of the unsaturated lactone group.

2898

Availability of synthetic media for streptococci.

FRANCES KRASNOW, HELEN B. RIVKIN and MARGARET L. ROSENBERG. (Introduced by Wm. Gies).

[*From the Laboratory of Biological Chemistry of Columbia University at the College of Physicians and Surgeons, New York City.*]

Six hundred and seventy-one synthetic media were tested to determine their availability for streptococci. They may be grouped into three series: (1) those testing the availability of carbon compounds (carbohydrates and related substances, glycerol, and organic acids such as lactic, malic, tartaric and citric); (2) those testing the availability of nitrogen compounds (the common amino acids, caffeine, betaine, urea, and inorganic ammonium salts such as $(NH_4)_2CO_3$, NH_4Cl, $(NH_4)_2\ HPO_4$); (3) those testing the availability of some inorganic substances (compounds of Ca, Na, K, and Fe, and S). Most of the media were those previously used for other organisms by different in-

vestigators. The composition of the media ranged from water solutions of single substances to combinations of ten different materials.

Estimate of Growth	1st Transplant Subcultures						2nd Transplant Subcultures					3rd Transplant Subculture				4th Trans. Subcultures			5th Tr. Subcult.		6th Tr. Subcult.
	1	2	3	4	5	6	1	2	3	4	5	1	2	3	4	1	2	3	1	2	
4+	103	66	29	19	11	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3+	192	103	80	56	33	23	0	4	2	1	0	0	0	0	0	0	0	0	0	0	0
2+	85	98	80	61	52	29	6	6	2	1	1	0	0	0	0	0	0	0	0	0	0
1+	52	45	42	36	41	31	81	34	27	25	21	0	0	1	1	0	0	0	0	0	0
Several Colonies	9	16	13	5	8	4	72	44	31	11	8	1	1	0	0	0	0	0	0	0	0
Total No. of media showing growth	441	328	244	177	145	93	159	88	62	38	30	1	1	1	1	0	0	0	0	0	0
Percent of media showing growth	66.5	49.7	39.3	28.6	22.0	14.1	25.6	13.3	9.4	5.7	4.5	0.1	0.1	0.1	0.1	0	0	0	0	0	0

Four hundred and forty-one permitted the streptococci to remain viable through one or more transplants. While it would seem from this, perhaps, that it is a comparatively simple matter to manufacture synthetic mixtures which will permit the growth of these organisms, our results indicate a marked deficiency in all of these media. The significant details may be summarized in the following table in which the figures recorded indicate the number of media showing living organisms in the various subcultures, as explained below:

The symbols, 4+, 3+, 2+, 1+, and the phrase "several colonies," are used to indicate the amount of growth; 4+ denotes the maximum. The tests on each medium included six transplants. The one from the "test suspension" (consisting of streptococcus growth removed from infusion agar with a spatula and suspended in 0.9 per cent NaCl solution) to the synthetic medium is designated as the first transplant; the five others are successive transplants at 24 hour intervals from synthetic medium to synthetic medium. The subcultures are transfers from synthetic medium to infusion agar in order to ascertain whether there were live organisms in the synthetic medium. Each successive subculture was made 24 hours after the one immediately preceding. Thus subculture 1 was made after 24 hours incubation of the transplant under consideration; subculture 2 after 48 hours, subculture 3 after 72 hours, etc.

The data in the table indicate: (1) a decrease in the per cent of media showing living organisms in successive transplants; (2) a very marked decrease in the number of "positives" in the successive subcultures of the first transplant series; (3) a change that is not so abrupt in the second transplant series; (4) only one medium that shows growth in the third transplant series; and (5) no medium showing growth in the fourth, fifth, or sixth transplants.

The media do not permit the physiological functions of this organism to continue normally; hence propagation decreases and finally ceases. Continued growth in the first two transplants may be due either to a small reserve store of necessary nutrient (nutrients?) or to the transfer of small amounts of some of the constituents of the conventional medium (infusion agar) on which the test culture was amassed.

2899

The effects of the iodides and of iodine on tuberculous guinea pigs.

H. W. BUTLER. (Introduced by C. W. Duval).

[*From the Department of Medicine, Tulane University, New Orleans, La.*]

The object of these experiments is to determine the probable detrimental effect of the iodine derivatives when administered to tuberculized animals. Similar experiments have been made in the past by different workers, but we deemed it advisable to repeat them.

Guinea pigs were treated with daily doses of three to five drops of a saturated solution of potassium iodide which would be equal to approximately 250 to 400 gr. daily to the human adult. One pig was treated with the tincture of iodine, receiving one drop daily, which equals about two drams daily to a 70 kilogram patient.

The first tuberculized guinea pig received its initial dose of potassium iodide nineteen days after injection. It had developed large inguinal glands at the site of injection and was considerably emaciated. It was given three drops of potassium iodide daily for a period of fifteen days. Marked improvement and diminution in the glands resulted. The medication was discontinued nine days, at the end of which time the pig relapsed. Potassium iodide therapy was resumed, the animal receiving five drops of saturated solution of potassium iodide daily for a period of twenty-five days. Marked improvement apparently occurred, but the guinea pig died seven days later and was autopsied. Generalized miliary tuberculosis was found.

Four additional guinea pigs were tuberculized, and ten days later all had developed large inguinal glands. One with a fistula, which seemed to be the most heavily infected, was selected as a control. Seven days later, or seventeen days after the injection, the pigs were separated and medication begun.

Two pigs were given four drops of potassium iodide by mouth, and one was given one drop of the tincture of iodine daily for fourteen days. The control pig was not treated. Twenty-one

days after the period of treatment, the pig receiving the tincture of iodine died, and autopsy revealed a generalized extreme tuberculous condition. Forty-five days after treatment was discontinued, the animals receiving the potassium iodide died and were autopsied. A generalized tuberculosis of extreme character was found. Sixty days after the last treated pig had died, the control pig died. Autopsy revealed a generalized tuberculosis, but much less marked than in the treated pigs.

The first pig treated with potassium iodide lived 76 days. The pig treated with the tincture of iodine lived 52 days. Another pig treated with potassium iodide lived 75 days, and the other, 76 days. The untreated tuberculized control pig lived 135 days.

2900

Eliminating confusion in colorimetric calculations.

A. R. ROSE.

[*From the Laboratory of the Prudential Insurance Company, Newark, N. J.*]

The clinical analyst is sometimes confused in his calculations from comparison colorimetric (or turbidimetric) readings, especially if circumstances force him to deviate slightly from the definite directions of any given method. To avoid irritation and loss of time the author keeps on his desk the following equation:

$$\frac{F}{R} \times S \times \frac{V_u}{V_s} \times \frac{D_2}{D_1} \times \frac{1}{V} = X$$

F, scale reading of the standard in millimeters, usually fixed at some definite point; R, scale reading of the sample analyzed; S, concentration of the standard, usually milligrams per 100 cc.; V_u, volume of the colored (or turbid) solution as matched against the standard; V_s, volume of the standard solution; D_1, volume of the sample (or the aliquot extract) taken for analysis; D_2, volume to which D_1 is diluted before developing color (or precipitating); V, the volume of D_2 used in developing the color; X, the concentration of the unknown in terms comparable to S.

i. e., milligrams per cc. if S is given as milligrams; multiply X by 100 if mg. per 100 cc. is required. In most routine clinical work all but the first two and last terms $\left(\frac{F}{R}S = X\right)$ are unity and out of mind. When not in unity it is more often a simple two times, a five times or a ten times dilution, but odd dilutions may occur, as, for instance, when the sample available becomes limited through accident or otherwise. The mathematics involved should not deter the technician from saving his case.

Iowa Branch

Children's Hospital, State University of Iowa, November 4, 1925.

2901

Studies in glandular fever (infectious mononucleosis).

F. J. ROHNER, C. W. BALDRIDGE and G. H. HANSMANN.
(Introduced by Fred M. Smith).

[*From Departments of Internal Medicine and Pathology, College of Medicine, State University of Iowa, Iowa City, Iowa.*]

Acutely hyperplastic lymphatic tissue from many causes, for example, scarlet fever,[1] whooping cough, typhoid fever, secondary syphilis, etc., has been known to result in an absolute increase in lymphoid cells in the circulating blood. This study is based on cases of apparently specific lymphadenopathy called glandular fever,[2] a condition characterized by general enlargement of the lymphatic glands, an absolute mononucleosis and the presence in the blood of abnormal mononuclear cells.[3] Our study is based on the findings in fifty clinical cases of glandular fever or infectious mononucleosis and in fifty-five medical students in whom the symptoms were so mild as to be negligible but in most of whom there was evidence of glandular enlargement and mononucleosis. Glands were removed from six patients.

Our purposes are: (1) to determine the etiology, (2) to ascertain whether or not the pathological changes in the glands are characteristic, (3) to establish the origin of the abnormal mononuclear cells, (4) to determine whether the mononucleosis is a specific response to the etiological agent, a response peculiar to certain individuals, or a compensatory reaction in individuals in which the granulocytic apparatus is rendered impotent by the infection.

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[1] Tilestone, W., and Locke, E. A., *J. Inf. Dis.*, 1905, ii, 375.

[2] Pfeiffer, E., *Jahrb. f. Kinderh.*, 1889, xxix, 257.

[3] Sprunt, T. P., and Evans, F. A., *Johns Hopkins Hosp. Bull.*, 1920, xxi, 410.

(1) Blood cultures were done on fourteen cases, thirteen of which remained sterile. In one culture diphtheroids were obtained both from bouillon and blood agar plates. Glands removed from six patients were cultured and diphtheroids[4] were obtained in pure culture from four, one of which was removed six months after the acute symptoms had subsided. The organisms grew best in veal bouillon and four to seven days were required for clouding the media. One cc. of a five day old bouillon culture either subcutaneously or intraperitoneally in guinea pigs caused mild fever of three to five days duration but no special symptoms nor distinctive adenopathy. Organisms were not recovered from the lymph glands of injected guinea pigs. Injections of gland emulsion in guinea pigs and rabbits were without effect. Suspensions of organisms were not agglutinated by the serum of convalescent patients. Dark field illumination of tissue fluid from the excised lymph nodes showed no spirochetes nor other organisms. Seventy-four of the eighty-four mouth smears studied for Vincent's organisms were positive.

(2) Pathology—All of six glands studied were very similar both in gross and microscopic appearance. All were axillary glands and were removed from four days to six months after the onset of illness. The size varied from 2 to 3 cm. in diameter and the glands were soft and spongy. They were kidney shaped with distended white capsules and cut sections were uniformly gray and granular. Histologically, the architecture was for the most part discernable though there was much distortion by a marked lymphoid hyperplasia which compressed the sinuses and stretched the capsules. Some germinal centers were replaced by uniform lymphoid hyperplasia. Mitoses were present in the germinal centers and lymph sinuses. Many very large lymphoid cells with irregular nuclei were found in the lymphatic cords and sinuses. An occasional eosinophile was also seen.

(3) All types of abnormal mononuclear cells appearing in the blood stream were found in the lymph sinuses of the glands. Blood smears stained by the method of McJunkin showed only a small percentage of the abnormal mononuclear cells to have the staining characteristics of endothelial cells.

The mononucleosis seemed to be directly dependent on the lymphoid hyperplasia. The lymphoid hyperplasia was very much

[4] Coon, H. M., and Thewlis, E., *Wis. Med. J.*, 1922, xxi, 191.

more marked and generalized than is usually seen in a localized infection with drainage to regional nodes as, for example, in streptococcic sore throat. The epidemic nature of the condition is also in favor of a specific etiological agent. In order to demonstrate the ability of the granulocytic apparatus to respond, four patients were given a foreign protein (typhoid vaccine) intravenously. All patients responded with a leukocytosis, the increase being entirely confined to the polymorphonuclear leukocytes.

2902

Chronic benzol poisoning.

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(Introduced by Fred M. Smith).

[*From Departments of Internal Medicine and Pathology, College of Medicine, State University of Iowa, Iowa City, Iowa.*]

Our purposes were to verify in human pathology, the morbid anatomical changes described in animals poisoned with benzol, and to observe the effects of benzol medication on patients with chronic myelogenous leukemia.

The first studies were on a patient occupationally exposed to the fumes from a vat of benzol from January 6, 1925, to March 20, 1925. The symptoms, findings and course including a terminal sepsis were typical of chronic benzol poisoning. Entrance to the hospital was on May 27, 1925, and death on June 7, 1925.

Blood: R.B.C. 860,000; W.B.C. 1400; Hb. 20 per cent. Differential. Polymorphonuclear neutrophiles 13 per cent; lymphocytes 48 per cent; endothelial leucocytes 39 per cent. No normablasts were seen. Blood platelets 70,000. Coagulation time 9 minutes. Bleeding time 13 minutes.

Necropsy showed hemorrhages into the skin, mucous membranes and meninges. The lungs had the gross appearance of broncho-pneumonia. Microscopically the alveoli were plugged with fibrin which contained many organisms but no inflammatory cells. Numerous areas of focal necrosis were present in the liver. Organisms were plentiful in these areas but no inflammatory

cells could be found. The bone marrow contained very few cells of any type and there was no evidence of active formation of either red or white corpuscles. No megacaryocytes were seen.

Our findings conform clinically to those of reported cases,[1] and confirm, in the human, numerous morbid anatomical changes observed in experimental animals, namely:

1. Leucopenia.

2. Aplastic anemia.[2] Our own case did not verify the destruction of adult forms, as reported by Selling, since there was no excess of iron pigment in the tissues.

3. Thrombocytopenia.[3]

4. Aplasia of bone marrow, *i. e.*, replacement of the erythropoetic, leucopoetic and platelet forming elements by fat.

5. Absence of inflammatory cell response to infection.[4]

In addition our observations seem to warrant the conclusion that endothelial cells, both circulating and fixed, are not especially damaged by benzol.

The following facts have been quite well established clinically and experimentally and are in agreement with the results seen in twenty-one of our cases of chronic myelogenous leukemia which were treated with benzol.

1. A leucopenia is the earliest index to chronic benzol poisoning.[5] A prompt fall in the number of cells of myelocytic origin was noted in all cases, the more immature forms disappearing from the circulation first.

2. Injury to the erythroblastic elements is the last to appear and the last to disappear.[2] In most of the cases the fall in red blood corpuscles was very gradual and in the interval between admissions the leucocytes promptly rose while the erythrocytes lagged considerably behind.

3. There is marked variation in personal susceptibility to benzol.

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[1] Santesson, *Arch. f. Hyg.*, 1897, xxxi, 336.
[2] Selling, L., *Ziegler's Beitrage*, 1911, li, 576.
[3] Duke, W. W., *Arch. Int. Med.*, 1913, xi, 100.
[4] Winternitz, M. C., and Hirschfelder, A. D., *J. Exp. Med.*, 1913, xxvii, 657.
[5] Hamilton, A. P., *J. Amer. Med. Assn.*, 1922, lxxviii, 627.

Case	Tot. dosage of benzol in minims.	Period of administration.	Leucocytes		Erythrocytes		Result
			Before	After	Before	After	
1	3495	47 days	340,000	11,200	5.6m	6.1m	Symptomatically improved
2	555	25 days	292,000	300	2.1	1.0	Purpura and death
3	7104	3 1/3 yrs.	430,000	11,600	3.3	.8	No untoward symptoms

4. The effect of benzol on the bone marrow persists after withdrawal of the drug.

Benzol was routinely discontinued when the leucocyte count reached 30,000 but in many cases these cells continued to fall to a level well below normal.

The action of benzol in these cases, though parallel to the action on normal subjects, is not in itself conclusive because the patients were diseased and many of them received radium or Roentgen ray or both simultaneously with the benzol.

2903

Fertility of the white rat on purified rations.

AMY L. DANIELS and MARY K. HUTTON.

[*From the Department of Nutrition, Child Welfare Research Station, State University of Iowa, Iowa City, Iowa.*]

It has been observed that animals (rats) fed exclusively on milk[1] or on purified rations made to simulate milk[2] seldom reproduce. In those instances where reproduction has occurred, few young were born and only a small percentage have survived the suckling period. The failure in the reproduction function of

[1] Daniels, A. L., and Hutton, M. K., *J. Biol. Chem.*, 1925, lxiii, 143.

[2] The purified ration consisted of casein 18 grams; lard 23 grams; butter 5 grams; cod liver oil 2 grams; cornstarch 47 grams; NaCl 0.514 grams; K_2HPO_4 2.587 grams; $NaH_2PO_4.4H_2O$ 1.172 grams; magnesium citrate 0.76 grams; $CaCl_2.H_20$ 2 grams; iron citrate 0.75 grams; potassium iodide 2 per cent solution, 0.4 cc.; alcoholic extract of 22 grams of wheat embryo.

these animals has been attributed to a vitamin deficiency,[3] the vitamin in question being an ether-soluble complex associated with certain fats, which is present in green leaves, egg yolk, and many natural foods. Another group of investigators working with milk[4] and so called purified mixtures in which milk has formed the basis of the ration have postulated that milk is deficient in certain essential inorganic substances. Rats fed on milk to which given amounts of iron, iodine, sodium silicate, sodium fluoride, manganese sulphate and aluminum potassium sulphate were added produced through six successive generations an average number of normal young. With combinations of two or three, or, when single salts were fed, the results were confusing, so that no conclusions could be drawn as to whether all or part of these salts were essential. When, however, these salts were added to a purified ration, in which the fat component consisted of 23 per cent lard, 5 per cent butter fat and 2 per cent cod liver oil, all animals failed to reproduce.

In order to determine whether the reproductive failure on our purified ration is due to a vitamin or inorganic deficiency we have repeated our experiments with purified rations, using (1) larger amounts of those salts which were effective in correcting the deficiencies of milk, and (2) substituting, in some cases, cotton seed oil for the lard, and in others different amounts of butter in place of an equivalent amount of lard. We have also extended our experiments with milk, testing the effect of the ether extract of lettuce as well as the ash of various foods which have been demonstrated to be potent in correcting the sterility in our milk fed rats.

The addition of the ash of soy beans, lettuce, and yeast respectively, to milk in amounts equal to those furnished by the non-incinerated foods which had been effective in overcoming the sterility on the milk diets, resulted in the birth of normal young. Two generations of rats fed these milk modifications have been successfully raised. These results confirm our earlier

[3] Mattill, H. A., and Conklin, R. E., *J. Biol. Chem.*, 1920, xliv, 137; Mattill, H. A., and Stone, N. C., *J. Biol. Chem.*, 1923, lv, 443; Mattill, H. A., Carman, J. S., and Clayton, M. M., *J. Biol. Chem.*, 1924, lxi, 729; Sure, B., *J. Biol. Chem.*, 1923-24, lviii, 681, 693; Evans, H. M., and Bishop, K. S., *J. Am. Med. Assn.*, 1923, lxxxi, 889; *Anat. Rec.*, 1924, xxvii, 203.

[4] Anderegg, L. J., and Nelson, V. E., *J. Ind. and Eng. Chem.*, 1925, xvii, 453; Daniels, A. L., and Hutton, M. K., loc. cit.

findings, and indicate that milk is not lacking in the reproductive vitamin but is low in certain essential inorganic constituents.

On the other hand when lettuce ash, and larger amounts of those salts which were effective in correcting the deficiency of milk were added to our purified mixture, the animals failed to reproduce; whereas the addition of the ether extract of lettuce (0.3 gram per rat per day) to these rations, or the substitution of 23 per cent butter fat or cotton seed oil for the lard in our purified ration, resulted in the production of young at a comparatively early age. Sixty-four per cent of these have been reared through the suckling period. It would appear, therefore, that failure to reproduce on our former purified ration was due to a lack in the reproductive vitamin.

When yeast was used as a source of Vitamin B in the purified mixture containing an adequate amount of butter, lettuce extract, or cotton seed oil the second generation at two months of age was of average size (124 grams and 159 grams respectively in two groups) while the young of mothers receiving a similar purified ration in which a clear alcoholic extract of wheat embryo was used as a source of the antineuritic vitamin weighed only 44 grams (average of 8) at two months of age. The marked difference in the size of these two groups cannot be explained by the amount of Vitamin B furnished in the two rations, since in the latter the animals were given daily 100 cc. of the wheat germ extract in addition to that incorporated in the food. It seems probable that yeast carries essential inorganic substances and in studies aimed to determine the potency of certain salts some other source of Vitamin B must be used.

2904

Carbohydrate utilization during amytal anesthesia.

H. M. HINES, J. D. BOYD, and C. E. LEESE. (Introduced by J. T. McClintock).

[*From the Department of Physiology, State University of Iowa, Iowa City, Iowa.*]

Page[1] has shown that anesthesia produced by amytal is not associated with the hyperglycemic changes which usually accompany anesthesia. Numerous investigators have employed this anesthetic in experiments involving various phases of carbohydrate metabolism.

A comparison has been made of the response to intravenous glucose administration in six dogs with and without amytal anesthesia. The authors[2] have previously observed a marked constancy in the response of any one animal to repeated glucose injections. The amytal was administered in doses of 60 mg. per kilo body weight intraperitoneally or 100 to 110 mg. per kilo body weight subcutaneously. The depth of the narcosis during the course of the experiment varied in different animals from light to deep surgical anesthesia. The method employed for studying the response was the same as previously described.[2] A thirty per cent solution of glucose was injected intravenously by means of a Woodyatt pump at a rate of 4 grams per kilo body weight per hour for several hours. Observations of blood and urine sugar, urine volume, hemoglobin percentage, CO_2 content and pH of plasma, respiratory quotient and heat production (indirect calorimetry) were made on numerous occasions before, during and following the injection.

Each animal showed greater hyperglycemia, a marked increase of glycosuria and slightly greater fall of pH than was observed in control experiments on the same animal. Respiratory data vary but little from the controls. The average percentage of injected glucose excreted in the urine during and following the injection was 32.7 per cent in the animals with amytal and 18.6 per cent in the same animals without anesthesia.

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[1] Page, I. H., *J. Lab. and Clin. Med.*, 1923, ix, 194.

[2] Boyd, J. D., Hines, H. M., and Leese, C. E., *Am. J. Physiol.*, 1925, lxxiv, 656.

Average Blood Sugar Values for the 6 Animals.

	Without Amytal.	With Amytal.
Before injection	.099	.098
15 min. after injection began	.322	.400
1 hr. after injection began	.351	.468
2 hrs. after injection began	.353	.455
4 hrs. after injection began	.430	.453
30 min. after injection ended	.108	.155

Average Blood Plasma pH and CO_2 Content.

	Without Amytal.	With Amytal.
CO_2 before injection	49.0	50.6
CO_2 end of injection	40.0	38.6
pH before injection	7.357	7.365
pH end of injection	7.317	7.265

These results would indicate that certain phases of carbohydrate metabolism are disturbed in amytal anesthesia and that data obtained with its use should be interpreted accordingly.

Minnesota Branch

University of Minnesota, November 4, 1925.

2905

The anti-rachitic properties of breast milk.

CORNELIA KENNEDY (by invitation) and L. S. PALMER.

[*From the Section of Animal Nutrition, Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.*]

The cause of rickets in breast-fed babies has not been satisfactorily explained. It is not due to deficient phosphorus or calcium in the milk, as Von Meysenburg,[1] Von Meysenburg and DeBuys[2] have shown that the milk consumed by rachitic infants is no lower in its phosphorus or calcium than that received by normal infants. Courtney,[3] making a similar finding as regards K and Ca, points out that the diets of the mothers were deficient in fresh fruits, vegetables, and milk.

Contrary to the finding of Lesné and Vagliano[4] that breast milk does not have anti-rachitic properties we have found that it may be strongly anti-rachitic. While we can not state definitely that the anti-rachitic factor is present in breast milk only, as it is present in the food of the mother, we have found that a diet containing an ample supply of green vegetables, fruits, eggs, and milk, in addition to a small daily dosage of cod-liver oil, the fat of breast milk is markedly anti-rachitic.

The milk fat used was obtained from two sources; one a composite fat from the milk of three mothers on the same diet and one a fat from the milk of one mother. The first sample was fed at a level of 15 to 20 per cent of the food intake to three rats with severe rickets. One of the rats refused the fat, the other two took it for three days and on autopsy showed good line

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[1] Von Meysenburg, L., *Am. J. Dis. Child.*, 1922, xxiv, 200.

[2] DeBuys, L. R., and Von Meysenburg, L., *Am. J. Dis. Child.*, 1924, xxvii, 438.

[3] Courtney, A. M., *Am. J. Dis. Child.*, 1923, xxvi, 534.

[4] Lesné and Vagliano, *Compt. rend. soc. biol.*, 1924, xci, 143.

tests.[5] The second sample was fed to six rachitic rats at levels ranging from 5 to 25 per cent of the food intake. It was found that a fat level of at least 8 per cent was needed to produce a distinct line test. This may explain the results of Lesné and Vagliano, who fed only 5 per cent of the ether extract of mother's milk. We are continuing this work to ascertain if the factor is present in milk only as it is present in the food of the mother.

2906

A test of indolinones as agents for prevention and cure of polyneuritis.*

ROSS AIKEN GORTNER, L. S. PALMER, and SELMER J. DAHL.

[*From the Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.*]

Substances which have been reported as having a specific action in relieving experimental pigeon polyneuritis include various hydroxypyridins,[1] thyroxin and pilocarpine,[2] β-propylindolinone,[3] tyramine,[4] and histamine,[5] although the antineuritic effect of histamine has been questioned.[6] Attempts to prevent polyneu-

[5] McCollum, E. V., Simonds, N., Shipley, P. G., and Park, E. A., *J. Biol. Chem.*, 1922, li, 41.

* Published with the approval of the Director as paper No. 567, Journal Series. Minnesota Agricultural Experiment Station. The results presented formed a portion of the thesis of Mr. Dahl for the M.S. degree, University of Minnesota, 1924.

[1] Williams, R. R., *J. Biol. Chem.*, 1916, xxv, 437; *ibid*, 1916, xxvii, 431; *ibid*, 1917, xxix, 495.

[2] Dutcher, R. A., *J. Biol. Chem.*, 1919, xxxix, 63.

[3] Dutcher, R. A., Holm, G. E., and Bierman, H., *Science*, N. S., 1920, lii, 589.

[4] Abderhalden, E., *Arch. physiol.* (Pflüger's), 1923, cxcviii, 571; Lipschitz, W., *Z. physiol. Chem.*, 1923, cxxiv, 194.

[5] Abderhalden, E., loc. cit.

[6] Cf. Koskowski, W., *Arch. Intern. pharmocodynamie*, 1922, xxvi, 367.

ritis by histamine,[7] and pilocarpine[8] have not been successful, although success has been reported[9] with trimethyluracil and 4-phenylisocytosin.

The similarity of structure of indolinone and thyroxin[10] suggested a further study of their anti-neuritic properties, on the theory that the anti-neuritic vitamin may owe its properties to keto-enol isomerism as first suggested by Williams.[11]

The β-methylindolinone was synthesized by the method of Brunner[12] and purified by recrystallization from hot ligroin. Two lots of crystals were used, one melting at 110°-111° C. and the other at 117° C. The β-propylindolinone had been prepared previously in this laboratory. The crystals melted at 110° C. (uncorrected).

The vitamin B-like nature of these substances were tested by Seidell's[13] constant weight method and by administrating them to birds which had developed acute polyneuritis on a polished rice diet. In the tests by Seidell's method the birds were first brought into weight equilibrium by a 200 mg. dose of activated solid on alternate days, the diet being polished rice and water, *ad lib.;* the substance to be tested was then given in suitable doses in place of the vitamin B preparation. A second period of vitamin B feeding concluded the test. The curative tests were conducted in the usual manner.

Five groups of pigeons, each containing 3 birds, received β-methylindolinone on alternate days in doses equivalent to 3, 5, 10, 15, and 50 mg. daily, respectively. In all cases a marked decrease in weight occurred during the feeding of indolinone for periods varying from 6 to 18 days while either a cessation of this decline or an increase in weight accompanied the resumption of the vitamin B. One case of polyneuritis occurred during the indolinone feeding at the 10 mg. level. The same general results accompanied the feeding of β-propylindolinone in amounts equiv-

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[7] Koskowski, loc cit.

[8] Arloing, F., and Dufourt, A., *Compt, rend. soc. biol.*, 1923, lxxxviii, 775.

[9] Williams, R. R., *J. Ind. Eng. Chem.*, 1921, xiii, 1107.

[10] Kendall, E. C., *Ind. Eng. Chem.*, 1925, xvii, 525.

[11] Williams, R. R., *J. Biol. Chem.*, 1916, xxv, 437.

[12] Brunner, K., *Monatsch.*, 1897, xviii, 527.

[13] Seidell, A., *U. S. Public Health Report*, No. 262 (1922). We are grateful to Dr. Seidell for the activated solid (Fuller's earth containing adsorbed vitamin B) used in our tests.

alent to 15 mg. daily. Only 3 birds were used for this compound. When thyroxin was fed at 2 mg. daily doses, the decline in weight was very rapid, as though the stimulation of metabolism accentuated the vitamin deficiency.

In the curative tests, which were limited to only a few birds, temporary relief from acute polyneuritis was obtained with β-methylindolinone after ingestion of 100 mg. in two doses. This lasted for several days. Similar tests with the other compounds were inconclusive, due to the limited number of cases observed.

2907

Control of magnitude and direction of the continuous bioelectric currents associated with organic polarity.

E. J. LUND.

[*From the Zoological Station, Naples, and the Laboratory of General Physiology, University of Minnesota, Minneapolis, Minn.*]

In previous work it has been shown that the apical ends of isolated stem of Obelia colonies are electropositive (galvanometer circuit) to middle or basal regions of the same stem.[1] These differences of electric potential are maintained and therefore a continuous output of electrical energy occurs under normal conditions of life in sea water. The processes upon which this output of energy depends can be inhibited in a perfectly reversible manner by cyanide, ether and chloroform.

Tissues of the stem, the electric potentials of which have been repeatedly and reversibly decreased, retain their normal capacity for growth and regeneration and also their normal sequence when removed to pure sea water.

The normal electrical polarity of the stem as a whole is a result of the inequality of the P.D.s across the ecto-endoderm of the apical (young) and basal (old) ends of the stem. The ecto-endoderm of the apical end is usually the seat of the highest P.D.

[1] Lund, E. J., *J. Exp. Zool.*, 1925, lxi, 155.

From this it follows that removal or reversal by means of cyanide, ether and chloroform, of the electrical polarity of the stem as a whole does not necessarily involve a reversal of the direction of the P.D. across the ecto-endoderm but merely an unequal decrease in its P.D. at apical and basal ends.

Alcock[1] and recently Csillag[2] have shown that the P.D. in the frog's skin can be reversibly decreased with suitable concentrations of ether and chloroform applied to both sides of the skin. Experiments by the writer show that cyanide also decreases reversibly this P.D. But in no case has a reversal in the direction of P.D. across the skin been obtained.

2908

An improved portable calorimeter.

J. F. McCLENDON.

[*From the University of Minnesota School of Medicine, Minneapolis, Minn.*]

The apparatus consists of a six liter spirometer with recording drum, showing liters on the ordinate and minutes on the abscissa. Below the spirometer is a six liter glass museum jar, which in operation contains one liter of 0.1 *M* $Ba(OH)_2$ solution, containing about 0.1 mol of $BaCl_2$ and 0.1 gm. phenolphthalein, and a motor-driven centrifugal pump which sprays this solution through the air in the jar at a very rapid rate. A single motor drives the pump and the recording drum.

The apparatus is filled with oxygen at the start and a mask is attached over the patient's face, one tube from which leads down into the jar below the surface of the solution; a second tube leads from the spirometer through a valve and T-tube to the mask, and a third tube passes from the jar into the spirometer to complete the closed circuit. The motor is started, writing a base line on the drum. The mask is attached to the patient's face, leaving the

[2] Alcock, N. H., *Proc. Roy. Soc.*, Ser. B., 1906, lxxvii, 159.

[3] Csillag, E., *Arch. f. Exp. Path. Pharm.*, 1924, ci, 296.

T-tube open so that there is free communication with the outside. At the end of an expiration, a stopper is quickly inserted into the T-tube, and at the moment the phenolphthalein is decolorized, this stopper is removed. Two and four-tenths liters of CO_2 are exhaled during the period and the oxygen is calculated from the record on the drum in the usual manner, using the temperature of the water in the spirometer as the oxygen temperature. The vapor tension of the water is subtracted from the barometric pressure. The advantage of the apparatus over the other portable calorimeters is that the respiratory quotient is determined.

2909

The dissociation constants of some indicators for the determination of the pH by the Duboscq colorimeter.

R. S. HEGGE. (Introduced by J. F. McClendon).

[*From the University of Minnesota Medical School, Minneapolis, Minn.*]

Indicator	pH at half color	Dissociation constant
4-6-dinitro-guaiacol	3.4	4×10^{-4}
Pinacyanol	3.7	2×10^{-4}
Ortho-chrom-T	6.7	2×10^{-7}
1-3-5-trinitrobenzene	12.8	1.6×10^{-13}

Dnitro-benzoylene-urea showed two dissociation constants and was therefore unsuitable. One of these dissociation constants was about 10^{-12}.

2910

The dissociation constant of orthocresol-tetrachlorophthalein.

MYRTLE HUNDLEY (by invitation) and J. F. McCLENDON.

[*From the University of Minnesota Medical School, Minneapolis, Minn.*]

Since colorimeters of the Duboscq type are usually found in medical laboratories, it seemed desirable to add to the list of indicators which can be used in such instruments for the determination of the pH. Dr. Ralph T. K. Cornwell kindly sent us specimens of orthocresol-tetrachlorophthalein and iso-orthocresol-tetrachlorophthalein described by Orndorff and Patel,[1] and E. L. Arnold.[2]

We found the dissociation constant of the first named indicator to be 1.78×10^{-9}, showing a fifty per cent color change at pH = 8.75. In this study we used the borate buffers of Palitzsch, checked them against the hydrogen electrode, and determined the dissociation by the amount of color measured by the Duboscq colorimeter (assuming 100 per cent dissociation in a 0.1 *N* NaOH solution). The indicator is only very slightly soluble and therefore has to be used in rather a deep layer of fluid.

The other indicator is even less soluble and has two dissociation constants close together.

2911

Some hydrogen electrode measurements on normal blood.

J. F. McCLENDON and HENRY ULRICH (by invitation).

[*From the University of Minnesota Medical School, Minneapolis, Minn.*]

Owing to the fact that the pH of blood is usually determined colorimetrically, it seems desirable to compile all the hydrogen electrode measurements that we can get. Therefore, the following measurements, although not very recent, are here given since

[1] Orndorff and Patel, *J. Am. Chem. Soc.*, 1925, xlvii, 863.

[2] Arnold, E. L., *ibid.*, 1924, xlvi, 489.

they have not been previously published. The subjects were patients from the University Hospital and all but one were males.

Date	Name	pH of blood at 20°	Disease
October 7, 1916.	Bloomquist	7.40	— —
October 7, 1916.	Rotay	7.41	— —
October 14, 1916.	Rotay	7.45	— —
October 20, 1916.	Carlson	7.35	— —
October 20, 1916.	Charlotte Clarine	7.36	Polycythemia
October 25, 1916.	Watson	7.50	— —
October 25, 1916.	Charlotte Clarine	7.40	
October 25, 1916.	Keene	7.45	Nephritis
November 5, 1916.	Sweeney	7.45	Nephritis

2912

Comparative solubilities of creatinine and guanidine picrates.

GRACE MEDES. (Introduced by J. F. McClendon).

[*From the University of Minnesota Medical School, Minneapolis, Minn.*]

Reports of the appearance of guanidine in the urine, in cases of tetany, have depended upon the precipitation of guanidine picrate from urine, after it has been freed of inorganic salts. No attempt is made apparently to remove creatinine. Guanidine picrate could be obtained free from creatinine picrate by this method only in case the former was much more highly soluble than the latter in the solvents (water and 50 per cent alcohol) employed. To test this point a comparative study of the solubilities of guanidine and creatinine picrates in water and 50 per cent alcohol was made.

Solubilities (Gram solute: Grams Solution).

Temperature degrees	Water Guanidine Picrate	Creatinine Picrate	Temperature degrees	50 per cent alcohol	
				Guanidine Picrate	Creatinine Picrate
92	1:115.7	1:63.6	74	1:60.2	1:32.2
78	1:199.1	1:96.6	57	1:113.2	1:58.2
48	1:599.4	1:240.3	37	1:252.2	1:132.8
32.5	1:1112.2	1:380.0	19	1:523.9	1:276.4
21.0	1:1648.4	1:549.2	8	1:830.1	1:447.0
7.5	1:2898.5	1:819.2			

Creatinine picrate is more soluble in water and 50 per cent alcohol than is guanidine picrate, and therefore cannot be removed quantitatively from a mixture of the two salts by extraction with either of these solvents.

2913

The growth in mass of the various regions of the body in the fetal period.

RICHARD E. SCAMMON.

[*From the Department of Anatomy, University of Minnesota, Minneapolis, Minnesota.*]

It has been pointed out by Calkins,[1] Calkins and Scammon[2] and others[3] that the growth of a large number of the external dimensions of the human body in the fetal period is directly proportional to the growth in total body-length, and that the probable values of these dimensions in this period may be expressed by the general formula:

$$D = aL \pm b \quad (1)$$

where "D" is the dimension in question, "L" is the total body-length, "a" is a constant in the form of a decimal fraction, and "b" is a second constant in the form of an absolute number. In accord with the law of developmental direction the "b" constant is positive for dimensions of the head and neck, negative for dimensions of the extremities and positive, negative or zero for measurements of the trunk, depending on their position.

If this law holds true for all external bodily dimensions the volumes of the major body-parts should bear a similar relation to the volume of the body as a whole, or:

$$P_v = aB_v \pm b \quad (2)$$

where "P_v" is the volume of any major body-part, "B_v" is the

[1] Calkins, L. A., *Anat. Rec.*, 1921, xxi, 47, and *Am. J. Obstet. and Gyn.*, 1922, iv, 109.

[2] Calkins, L. A., and Scammon, R. E., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 353.

[3] Akiba, T., *Fol. Anat. Japon.*, 1924, ii, 189.

total volume of the body, and "a" and "b" are constants as in (1). In spite of the small differences in the specific gravity of the various parts of the body in prenatal life, the same relation should be approximated by the weights of the body and its various parts.

It is possible to test these relations with a series of observations by Corrado[4] which has not been analyzed hitherto. These data include 137 observations on the weight of the head (and neck), and total body-weight for specimens ranging from 400 to 4000 gm. in body-weight. When these are averaged for 400 gm. intervals of body-weight the relation between head and body-weight (as determined by the method of averages, which means weighted for the number of cases in each interval) is:

$$HW = 0.23600\ BW + 79.4\ gm. \quad (3)$$

where HW is the weight of the head in grams, and BW is the weight of the total body in grams. The sum (without regard to sign) of the deviations of the observed 400 gm. range averages from the corresponding computed values is 118.0 gm., the unweighted mean deviation is 13.1 gm., and the mean deviation, weighted for the number of observations in each interval, is 11.1 gm.

The relation similarly determined for 137 observations on the weight of the trunk is:

$$TW = 0.48596\ BW - 29.8\ gm. \quad (4)$$

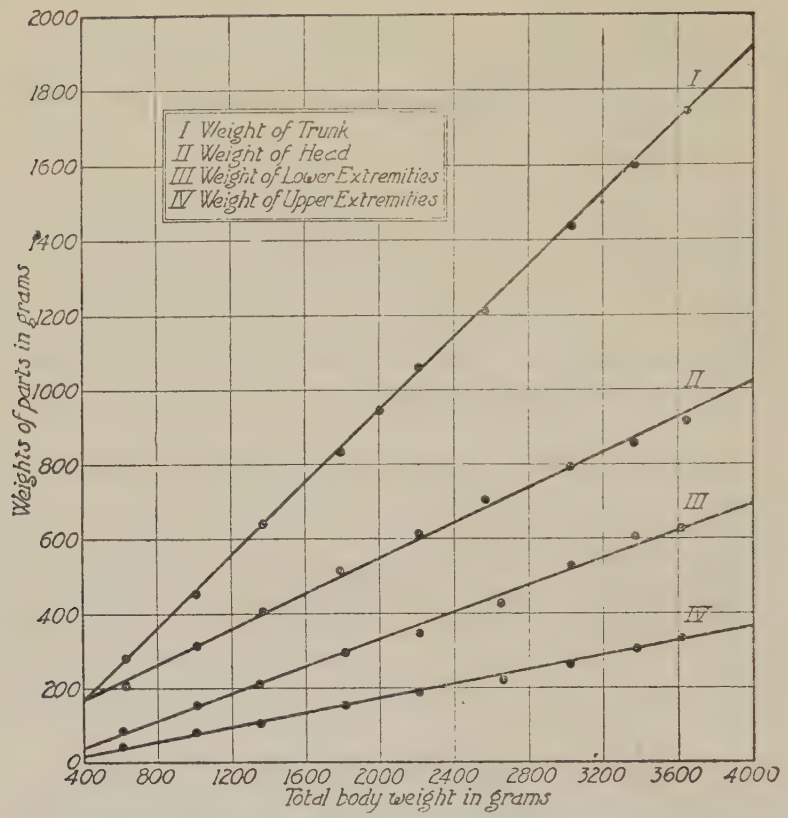
The sum (without regard to sign) of the deviations of the observed 400 gm. range averages from the corresponding computed values is 75.9 gm., the unweighted mean deviation is 8.4 gm., and the mean deviation, weighted for the number of observations in each interval, is 8.5 gm. These relationships are shown by curves I and II in the following figure.

A second set of data collected by Corrado is available for the study of ponderal growth of the extremities. There are 161 observations on the weight of the upper extremities of specimens having body-weights between 400 and 4000 gm. and the relation therefor, determined by the method noted above, is:

$$UEW = 0.09647\ BW - 22.7\ gm. \quad (5)$$

The sum (without regard to sign) of the deviations of the observed 400 gm. range averages from the corresponding computed values is 39.9 gm., the unweighted mean deviation is 4.3

[4] Corrado, G., *Gior. d. Ass. Napol. Med. et Natural.*, 1899, ix, 405.



A graph illustrating the relations of the weights of various major parts of the body to the weight of the body as a whole in the fetal period. The dots represent the observed averages for 400 gm. intervals of body-weight. The curves are drawn to the empirical formulæ given in the body of this article.

gm., and the mean deviation, weighted for the number of observations in each interval, is 4.3 gm.

The expression (based on the same number of cases) for the relation between the weights of the lower extremities and of the body is:

$$LEW = 0.18042\ BW - 31.1\ gm. \quad (6)$$

The sum (without regard to sign) of the deviations of the observed 400 gm. range averages from the corresponding computed values is 128.2 gm., the unweighted mean deviation is 14.24 gm., and the mean deviation, weighted for the number of observations in each interval, is 13.8 gm.

Curves III and IV of the accompanying figure show the relation of these computations.

These findings support those quoted above for the inter-relationships of the external bodily dimensions and indicate that the growths of the various major parts of the body in the fetal period are directly proportional to the ponderal growth of the body as a whole. The presence of a positive "b" constant in formula (3) for head weight and of negative "b" constants in formulæ (5) and (6) for the weights of the extremities offers further evidence of the action of the law of developmental direction in the fetal period.

Peking Branch

Peking University, China, October 8, 1925.

2914

A comparison of different urease preparations in the determination of urea.

SCHMORL M. LING. (Introduced by F. R. Dieuaide).

[*From the Chemical Laboratory, Department of Medicine of the Peking Union Medical College, Peking, China.*]

Since Takeuchi[1] discovered a "selective enzyme," urease, in soy bean (the seeds of Glycine hispida), more than fifteen methods have been developed for the preparation of urease solution or powder for the determination of urea in blood and in urine. Experience has shown that each has its own advantage and all have the same disadvantage.[2]

No attempt has been made to try all of these methods. However, it is worth while to determine which one gives the best results and which is, therefore, best adapted to routine work. At random, the methods proposed by Armstrong and Horton and by Folin and Wu were selected for trial and used to compare the extractions from Jack bean meal with 10, 5, and 2 per cent sodium chloride solution, and with distilled water (5 gm. meal in 100 cc. solvent). Since Sumner and his co-workers[3] believe that urease is a protein and since the vegetable proteins can be extracted by sodium chloride solutions of different strengths and by water, it is thought that extraction from Jack bean meal with sodium chloride solution of a certain strength may be of a high activity.

In all the experiments urease extracted with 5 or 2 per cent

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[1] Takeuchi, T., *Chem. Abstr.*, 1910, iv, 213.

[2] Ling, S. M., Urease, a bibliographic review, to be published.

[3] Sumner, G. B., Graham, V. A., and Noback, C. Y., Proc. Soc. Exp. Biol. and Med., 1924, xxi, 551; Sumner, J. B., and Graham, V. A., *J. Biol. Chem.*, 1925, lxii, 43.

sodium chloride solution or with distilled water, and the one prepared according to the directions of Armstrong and Horton seems to be more active in decomposing urea than the alcoholic extract prepared according to the method of Folin and Wu. The former recovers 99 per cent of urea while the latter recovers only 92 per cent. The one prepared with 10 per cent sodium chloride solution gives nearly the same result as the Folin and Wu urease solution, which may be explained by the depressing effect of sodium ion.[4]

An alcoholic extract of Chinese soy bean meal (yellow) was prepared according to the method of Folin and Wu and the activity of the enzyme urease was compared with that of a similar preparation of Jack bean meal. The fresh urease solutions from these two sources are efficient in decomposing urea to the same extent. After keeping them in the refrigerator for a week the activity of these two preparations is not diminished, but at the end of two weeks the former is able to decompose only half as much urea as before and about two-thirds as much urea as the Jack bean urease does, while the latter deteriorates slightly in this interval.

2915

A convenient apparatus for the determination of ferment action.

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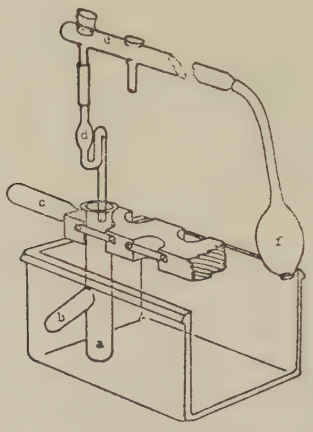
[*From the Department of Physiology, Peking Union Medical College, Peking, China.*]

In determinations of ferment action it is first of importance, that both ferment and substrate be brought to the same temperature before mixing. Secondly, the mixing of any series of tubes containing ferment and substrate should be carried out simultaneously. For this purpose, a test tube with a side arm (see figure) was constructed, so that (a) could contain the substrate and (b) the ferment. A series of these tubes is filled with the appropriate solutions, and placed in the holder (c), which ena-

[4] Onodera, N., *Biochem. J.*, 1915, ix, 575.



bles one to handle the entire series of tubes while in the water bath. When the desired temperature has been attained, the two components in every tube may be mixed at once by simply rotating the handle of (c).

In certain methods it is of importance that the non-digested substrate in the entire series of tubes be precipitated at the same time. The parts (d, e, f) are available for this purpose. Through the opening in (e) a sufficient amount of the precipitating-fluid is introduced with a pipette into the trap-tube (d) after which the openings in (e) are tightly stoppered. The end of tube (d) is introduced into the mouth of the test tube (a, b). At the appropriate time, pressure on the bulb (f), immediately followed by rotation of the handle (c), will effect immediate and simultaneous precipitation in all tubes.

In determinations of the trypsin-inhibiting power of serum the v. Bergmann-Gross-Fuld Method[1, 2] it is important that the trypsin should be placed in one limb of the test tube (a, b) and the substrate and serum in the other; if trypsin and serum are at first placed together, large errors may ensue in consequence of the trypsin being "fixed" by the serum in amounts depending upon the length of time during which the two substances are in contact before exposure to the substrate.

[1] v. Bergmann und Bamberg, *Berl. klin. Woch.*, 1908, 1396; u. Meyer, *ibid.*, 1673.

[2] Hedin, *J. Physiol.*, 1905, xxxii, 390; *Zeitschr. f. physiol. Chem.*, 1906-7, l, 497.

2916

Excystment phenomena in clonorchis sinensis.

E. C. FAUST and O. K. KHAW.*

Clonorchis sinensis is a digenetic trematode living in the bile passages of man and other mammalian hosts, particularly dogs and cats. The infection is found endemically only in the Far East. Although infection in reservoir hosts is common throughout the Sino-Japanese areas, that in man is confined almost entirely to one restricted area in Japan, to the southern half of Korea, to Kwangtung Province, China, and to Tonkin Province, French Indo-China. Cases found outside these endemic areas have without exception been traced back to them.

The Process of Encystment. The life cycle of Clonorchis involves a snail, *Bythinia striatula,* as first intermediate host and fresh-water fishes as second intermediate hosts. The second larval stage, the cercaria, attacks the fish, attempting to burrow under the scales and into the flesh. During this process it drops its tail and, after penetrating as far as it can into the new host, secretes a viscous fluid which gradually hardens to form a spherical cyst wall, *the inner (true) cyst capsule.* The presence of the encapsulated larva in the tissues of the fish, and the excretion of its waste products into the host's tissues causes the latter to lay down an outer false wall which is fibrous in nature and adheres firmly to the inner capsule. The process is comparable to the encystment of trichina larvæ that have migrated into the striped muscles of mammals.

Japanese investigators have found that various cyprinoid fishes are involved in this phase of the Clonorchis life cycle, but our studies have shown that practically any fresh-water fish which is exposed to the infection may serve as the host of the encapsulated larvæ. While the encysted larvæ are usually found some distance beneath the epidermis, a certain proportion fails to penetrate the superficial layers of the fish and becomes encapsulated either on the epidermis or attached to the under side of the scales. Here the larva obtains nourishment from the surrounding itssues of the host and increases in size, the elastic capsule enlarging to accommodate the growing larva. Our experiments have shown

* Contribution No. 65 from the Parasitology Laboratory. Department of Pathology, Peking Union Medical College, Peking, China.

that cysts which have just been formed are viable when introduced into an experimental mammal and that they remain viable in the tissues of the fish for several months following encystment.

The Phenomena of Encystment. The experiments reported in this communication have to do with the processes of encystment and the arrival of the larvæ in the bile passages of the mammalian hosts. The data were derived from two types of experiments, (1) *in vitro* and (2) *invivo.* The former were undertaken to determine under what circumstances excystment took place. The latter were employed in order to confirm the former and to ascertain the exact route of migration of the larvæ into the bile tracts. We employed viable cysts from the under side of the scales of the knife-fish, *Hemiculter kneri,* obtained from the Peking Market.

(1) IN VITRO EXPERIMENTS.

(a) *Effect of gastric juice on the cyst.* Dog's gastric juice (preserved in toluol) was allowed to work on the encysted larvæ at 26° C. and at 37° C. for various lengths of time. In this medium digestion of the outer wall and of the fish scales took place but the inner true capsule remained intact. The action was much more rapid at 37° C. than at 26° C. The larva within at first became activated but later became quiescent and died after four or five hours. In case the cyst capsule was ruptured through artificial pressure the larva died on immediate direct contact with the medium. Boiled gastric juice and the HCl fraction had no effect either on the outer wall or on the true capsule, although the larva died as in the previous experiments. Fresh (unpreserved) gastric juice had the same digestive effect on the outer wall and the fish scales as the preserved juice but was less lethal to the larvæ within, which survived until about the twelfth hour.

(b) *Effect of intestinal juice on the cyst.* When the cyst was first placed in intestinal juice, either fresh or preserved (reaction neutral) without the previous action of gastric juice, no digestion of the inner or of the outer cyst wall took place up to 22 hours. If the capsule was ruptured by mechanical pressure, the larva escaped and was able to live in the fresh juice from 1 to 3 hours, but was digested after that time. In preserved intestinal juice it died almost immediately after rupture of the capsule.

(c) *Fresh (unpreserved) gastric juice followed by fresh (unpreserved) intestinal juice.* When the cyst was first submitted to the action of fresh gastric juice at 37° C. for 4 hours and was then transferred to fresh intestinal juice, the outer wall was

digested in the former medium and on introduction to the latter medium the larva became greatly activated, so that in 20 minutes *it caused the rupture of the cyst wall,* wriggled out into the free intestinal juice, and was active up to three hours, when observation was discontinued.[1] The cyst capsule had been somewhat weakened by the action of the intestinal juice but remained intact. The process as we observed it differed, therefore, from that which Kobayashi (1917) described for Clonorchis, to the effect that the digestive juices of the stomach and of the intestine had no effect whatever on the cyst capsule. It also differed from Ciura's statement (1917) regarding the related form. *Opisthorchis felineus,* in which he believed the larva was entirely passive, and excystment depended entirely on the digestion of the hyaline cyst capsule by the intestinal juice, which left the larva lying free in the medium.

From this series of experiments we have concluded that fresh gastric juice dissolves and digests the outer cyst wall and the fish scales but does not digest the true cyst wall. The larva within remains viable up to 12 hours *in vitro.* Transfer to fresh intestinal juice weakens the true cysts capsule *pari passu* with increased activity of the larva, which causes the bursting of the cyst wall, through the opening of which the larva wriggles out. The freed larvæ appear to live comfortably for three hours or more in fresh intestinal juice..

(2) *In Vivo Experiments. In vivo* experiments were carried out on guinea-pigs and small puppies that had been fed on a meal of raw infected fish[2] from 5 to 72 hours previous to autopsy. In both series five hours after feeding the food mass with the cysts still remained in the stomach. The cysts had been freed from the scales and flesh, which had been partly digested. The cysts capsule in each case was intact and the larvæ viable. In both series, eight hours after feeding, most of the larvæ were still found in the stomach, although about 20 per cent had reached the duodenum, and of these latter a few had become excysted and were freely crawling about in the lumen. Likewise, in both series, after fourteen hours cysts remaining in the stomach were non-

[1] When bile in distilled water or in 0.5 per cent Na_2CO_3 was substituted for the intestinal juice there was no spontaneous excystment.

[2] In the case of the guinea pigs the flesh was made into a paste and smeared onto cabbage or spinach leaves; for the puppies the fish was fed in small pieces.

viable. Those in the duodenum were excysted; most of those found were attached to the mucosa of the duodenum near the opening of the common duct. After 22 hours the larvæ found in the duodenum were all attached to the mucosa and were massed in the vicinity of the opening of the common duct.[3] After 48 hours larvæ had migrated into the common duct; none were found in the duodenum. From the 72nd hour they were found passing into the bile passages. Larvæ were never found in the jejunum, the gall bladder or the pancreatic duct.[4] Only a portion of the viable larvæ which entered the duodenum actually excysted, and from our experiments we conclude that neither encysted nor excysted larvæ which passed into the jejunum or ileum were able to survive but were digested along with the food mass. Even in the duodenum it was necessary for the excysted larvæ to secure attachment to the surface of the mucosa in order to migrate into the common duct. It seems highly probable that only about 20 per cent of the viable encysted larvæ entering the body with the food mass excysted and that only about 5 per cent actually reached the common duct and the bile passages. These data favor the view that the migration into the bile passages is direct and does not involve previous passage through the portal vein which Lutz (1892-93) claimed was involved in the case of *Fasciola hepatica.*

2917

The influence of chaulmoogra on sulphur metabolism.

BERNARD E. READ.

[*From the Department of Pharmacology, Peking Union Medical College, Peking, China.*]

Further metabolism studies with Chaulmoogra oil have been undertaken with special reference to sulphur excretion. Estimations were made to find out whether the cyclic pentene nucleus of Chaulmoogric acid is excreted like phenol as the ethereal sulphate,

[3] It seems probable that this is a chemotactic reaction.

[4] As determined both by examination of the contents of these tracts and by scraping their epithelial linings.

and whether the findings concerning tissue breakdown, acidosis, and suboxidation could be further elucidated.

Rabbits and dogs placed upon standard diets, as previously described,[1] were treated with Chaulmoogra oil and by the ethyl esters of Chaulmoogra. The drugs were administered orally, subcutaneously, intraperitoneally, and intravenously. Estimations were made for the urinary excretion of inorganic sulphates and total sulphate's by Folin's method, and total sulphur by Benedict's method. From the results obtained there was calculated the amount of the ethereal sulphate and the amount of neutral sulphur excreted.

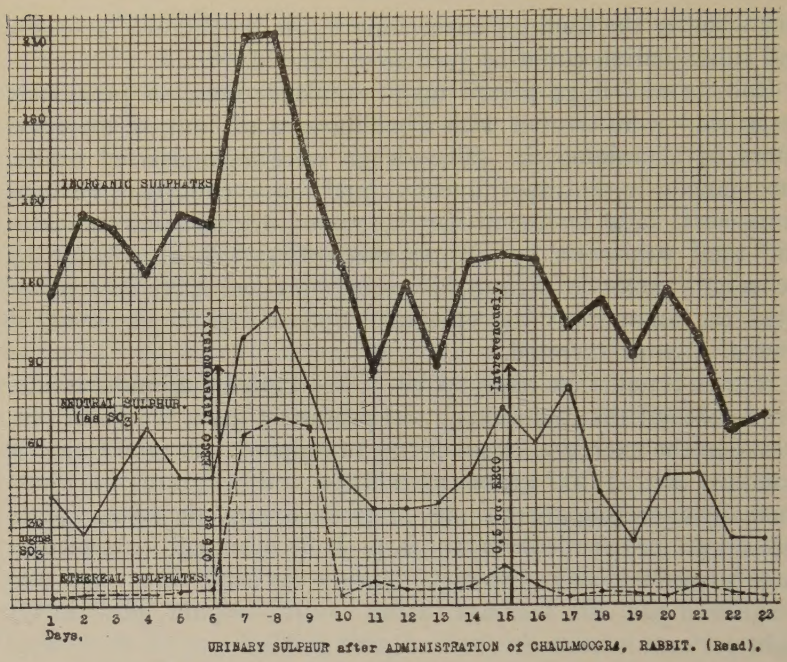
It was found that irrespective of the path of administration, large doses of the Chaulmoogrates produce a great temporary increase in the excretion of all forms of sulphur. A rabbit upon a standard diet of 100 grams of cabbage and 80 grams of wheat normally excreting about 6 mg. of ethereal sulphate (SO_3), after receiving 5 cc. of oleum hydnocarpi J. P. by mouth, excreted in three subsequent days 186 mg. During the same time there was a hundred per cent increase in the neutral sulphur, and thirty-eight per cent increase in the inorganic sulphates.

Repeated administration of the drug fails to produce the same result. Amounts less than normal for all of the sulphur compounds excreted are obtained. There is no increase in the ethereal sulphate excretion. The amount of the neutral sulphur decreases far below normal. A second dose of 5 cc. of oil given in the experiment quoted showed a reduction in the excretion of neutral sulphur to 30 per cent of the normal, on the sixth day. The inorganic sulphates dropped to 60 per cent of the normal on the seventh day.

Relatively smaller doses of this drug given to dogs yield similar results. The total sulphur excretion followed closely the curve for nitrogen excretion. Estimations of the total excretions over a period of several days showed little absolute increase in the total amount of ethereal sulphates and of neutral sulphur excreted. The inorganic sulphates showed definite increase. After one month's rest, further introduction of the drug again produced an increase in the excretion of ethereal sulphates.

The results indicate that the cyclic pentene compounds of the Chaulmoogric acid series are excreted by the organism in the

[1] Read, B. E., *J. Biol. Chem.*, 1924, lxii, 515.



URINARY SULPHUR after ADMINISTRATION of CHAULMOOGRA, RABBIT. (Read).

form of ethereal sulphates. There is tissue destruction, as indicated by the nitrogen metabolism, which probably takes part in the formation of ethereal sulphates. It is doubtful if the ethereal sulphates are formed by the utilization of sulphur from the exogenous metabolism. The endogenous metabolism is greatly increased and subsequently decreased. Suboxidation in the tissues may account for the absence of an increase in the excretion of ethereal sulphates after repeated dosage.

It is possible that the sulphur excretion is directly related to the values obtained for creatinine. However, this may be related to the large increase of sulphates resulting from the increased exogenous metabolism. The results indicate that large doses of Chaulmoogra oil, after a preliminary stimulation of and detoxication by the system, produce a state of decreased endogenous and exogenous metabolism, following tissue destruction, and a condition of suboxidation in the tissues.